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Determination of Optimal Dosage Regimen for Amikacin in Healthy Volunteers by Study of Pharmacokinetics and Bactericidal Activity

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The pharmacokinetics and serum killing curves of amikacin, which was administered by a 30-minute intravenous infusion of single doses of 7.5 mg/kg and then 15 mg/kg, were investigated in six healthy volunteers who received the two doses in a crossover study with a washout period of 20 days. The serum killing curves were determined for four bacterial species: *Escherichia coli*, *Serratia marcescens*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*. All strains were serum resistant, and the bactericidal activity was analyzed by separating the early phase (first 5 h) and the late phase (24 h) of the killing curve. For the early phase, the bactericidal activity was evaluated by correlating an index of surviving bacteria with amikacin concentrations. This methodology allowed determination of two parameters: the maximal effective concentration and the lowest effective concentration. For the late phase, the threshold values separating bacteriostatic and bactericidal activities were lower than 10 mg/liter for each strain. The concentration dependence of amikacin bactericidal activity was confirmed for *Escherichia coli* and *Enterobacter cloacae* and, to a lesser extent, for *Serratia marcescens* and *Pseudomonas aeruginosa*. Correlation of these data with amikacin pharmacokinetic data in volunteers indicated that a daily dose of 15 mg/kg may be effective in the treatment of *Escherichia coli* and *Enterobacter cloacae* infections. For *Pseudomonas aeruginosa* and *Serratia marcescens*, the partially time-dependent activity probably necessitates two daily administrations and combination with another antibiotic.

Over the past few years, pharmacokinetic principles have widely influenced the use of antibiotics. Advances in assay technology have allowed rapid measurements of drugs in blood and tissues and have provided detailed pharmacokinetic data for many antibiotics. This has allowed the scientific basis of dosage adjustment to be developed. To optimize the dosage further, the individual susceptibilities of the infecting bacteria must also be considered, together with pharmacokinetic optimization. This type of approach has been called dual individualization by Schentag et al. (10) and could allow inclusion of a fundamental parameter, i.e., the pharmacodynamics of antibiotics.

In current clinical practice, the antibiotic dosage is generally determined on the basis of drug pharmacokinetics, safety, and efficacy, as estimated by the MIC. However, the MIC is determined in vitro by using incremental increases in drug concentration. Since the concentration of a drug in the blood of patients is constantly changing, the use of a static concentration to estimate the efficacy of antibiotics is questionable. Thus, aminoglycosides such as amikacin are frequently given twice a day, even if their half-lives are known to be short, and in this case, concentrations in plasma may fall below the MIC.

In attempts to improve analysis of the time course of antibacterial effects on the growth of microorganisms, several investigators have investigated the in vitro killing curves of antibiotics with various gram-negative or gram-positive pathogens. The usefulness of the serum killing rate assay was recently validated in animal models of endocarditis and meningitis (2, 3, 15). Moreover, Van der Auwera et al. (12) showed that the bactericidal rate in serum better reflects the increase of antibiotic activity than bactericidal activity in serum does. Using this approach, Vogelman and Craig (13) have pointed out that antibiotics should be considered to have a concentration- or time-dependent bactericidal activity, depending on the time course profile of the bacterial killing rate. The validity of these findings has been confirmed in animal models, which provide important information for the management of antibiotic therapy in humans (14).

The purpose of the present investigation was to evaluate simultaneously, by a new method, the time course of the bactericidal activity and the pharmacokinetic profile of two amikacin doses in healthy volunteers. This new approach consisted of coupling the pharmacokinetics and pharmacodynamics data. The information that we obtained is of utmost importance for the correct and appropriate scheduling of antibiotic administration and provides improved guidelines for clinical trials.

MATERIALS AND METHODS

Volunteers. The protocol of this study was reviewed and approved by the Ethical Committee of the University Hospital of Nice. Six healthy volunteers were included in the study, after they gave their informed consent. We included three males and three females (ages, 23 to 31 years; weights 50 to 75 kg). The criteria used for exclusion of volunteers from the study were as follows: allergy to aminoglycosides, abnormal renal (serum creatinine of >125 μmol/liter) or hepatic (serum bilirubin of >1.0 mg/dl) functions, exposure to an antibiotic within the past month, history of hearing troubles, or convulsive disorders and pregnancy.

Administration of antibiotics. Each volunteer received the following doses of amikacin in a crossover study on separate days with a 3-week washout period: 7.5 mg/kg intravenously infused over 30 min in 150 ml of 5% glucose and then 15 mg/kg under the same conditions. The dose that was administered first was randomly allocated.

Blood samples were taken for determination of the drug concentration in serum before administration and at 0.5 (end of infusion), 0.75, 1, 1.5, 2, 3, 4, 6, 12, 18, and 24 h after

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infusion. The blood samples for determination of the bacterial killing rate were taken simultaneously with blood samples for drug concentration determination at 0 (before administration), 0.5 (end of infusion), 1.5, 3, 4, 12, 18, and 24 h.

Determination of amikacin level in serum. Levels of amikacin in serum were determined in each sample by a fluorescence polarization immunoassay (TDX; Abbot Laboratories, North Chicago, Ill.) or high-pressure liquid chromatography. Each sample was assayed in duplicate. The drug measurements were performed less than 1 week after the experiment. They were conserved at −40°C until they were analyzed.

Test strains. Four strains, i.e., *Escherichia coli* ATCC 25922 (MIC, 2 mg/liter), *Serratia marcescens* ATCC 8100 (MIC, 1 mg/liter), *Enterobacter cloacae* ATCC 23355 (MIC, 1 mg/liter), and *Pseudomonas aeruginosa* (MIC, 4 mg/liter) were selected for the study. All the strains were resistant to serum.

Killing rate of serum. A portion of each serum sample from each volunteer obtained during the pharmacokinetic tests was immediately frozen and used to measure the killing rate by a semiautomatic method (H. B. Drugeon, J. Caillon, M. E. Juvin, and J. L. Pirault, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 145, 1986). Each serum sample was diluted from 1/2 to 1/128 in Mueller-Hinton broth plus 5% albumin and inoculated with a suspension of the test strains. The bacterial inoculum was adjusted by nephelometry and reached approximately 10^6 CFU/ml at time zero. The bacteria were in the stationary phase. These samples were incubated at 37°C after dilution; and the surviving bacteria were counted after 1.5, 3, 5, and 24 h of incubation by using an automatic apparatus (Skatron). The data for each time point were distributed normally, and the variability of the method is less than 10% (6).

Pharmacokinetic study. The pharmacokinetic parameters were obtained by computing the experimental data with a two-compartment open model. The values for the peak level, the area under the serum concentration-time curve (AUC), the apparent volume of distribution, the total body clearance, and the elimination half-life for each patient at each test dose were calculated with APIS software (6). The AUC was computed by using a trapezoidal rule. The total body clearance was calculated as dose/AUC, and the elimination half-life was taken as 0.693/β, where β was determined by a log-linear least-squares fit. Statistical comparisons between the two doses were made by using the nonparametric Mann-Whitney test.

Expression of results. The number of surviving bacteria was expressed as $\log_{10}$ CFU per milliliter. The time course of bactericidal activity was analyzed by separating an early and a late phase of the killing curve. The early phase concerned the first 5 h of bactericidal activity, and we calculated the AUC of the surviving bacteria during this period as a percentage of the hypothetical area of the reference inoculum if no bactericidal activity occurred; this parameter was called the index of surviving bacteria (ISB) (Fig. 1). This calculation was performed at the third and fifth hours of incubation. A linear regression program (Statigraphic STSC) was then used to correlate bactericidal activity (AUC of the percentage of surviving bacteria) to the ex vivo amikacin concentrations in serum for each tested strain. This presentation of the data was used to estimate the maximum and minimum bactericidal concentrations, as follows. The maximal effective concentration (MEC) was obtained graphically by correlating the amikacin concentration with a value of 0% of surviving bacteria (AUC). The lowest effective concentration (LEC) was given by the amikacin concentration corresponding to the starting point of a decrease in the percentage of surviving bacteria (AUC).

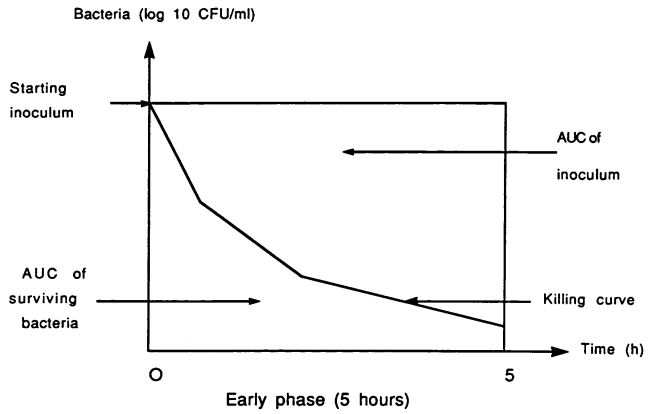

FIG. 1. Determination of ISB, where the percentage of surviving bacteria (ISB) = (AUC of surviving bacteria/AUC of inoculum) × 100.

In the late phase, the surviving bacteria (expressed as $\log_{10}$ CFU per milliliter) at hour 24 were compared with the amikacin concentrations in serum. This allowed determination of a threshold value under which regrowth phenomena appeared, the minimal concentration of regrowth (MCR).

RESULTS

Pharmacokinetic study. The within-assay and between-assay reproducibilities of the amikacin assay were ≤4.5 and ≤6.5%, respectively. The specificity of the test was excellent since the assay was not influenced by the presence of other antibiotics or drugs. Moreover, the volunteers did not receive any other therapy during the 15 days preceding the pharmacokinetic test.

The measured levels in serum and the derived pharmacokinetic data calculated after each amikacin dose are given as mean values in Table 1. Figure 2 shows the mean pharmacokinetic profiles after administration of each amikacin dose to the healthy volunteers. These data suggest that there is a good linearity of amikacin pharmacokinetics between the two tested doses. When the infused dose was doubled, the peak level of amikacin, which was reached at the end of the infusion, was more than twofold higher than that achieved

TABLE 1. Mean pharmacokinetics parameters after administration of amikacin to six healthy volunteers[a]

Parameter[b]	Amikacin dose	
	7.5 mg/kg	15 mg/kg
V_1 (liter)	11.00 ± 2.975	11.154 ± 1.776
k_{el} (h^{-1})	0.742 ± 0.246	0.62 ± 0.088
CL_T (liter · h^{-1})	7.60 ± 1.003	6.77 ± 0.552
$t_{1/2\beta}$ (h)	1.9 ± 0.211	2.37 ± 0.534
V_{area} (liter)	17.83 ± 2.253	17.96 ± 1.648
AUC (mg · h/liter)[c]	66.64 ± 15.742	154.53 ± 29.91

[a] None of the values was significant.

[b] Abbreviations: V_1, volume of central compartment; k_{el}, elimination rate constant; CL_T, total body clearance; $t_{1/2}$, elimination half-life; V_{area}, apparent volume of distribution; AUC, area under the curve.

[c] For comparison, AUCs must be normalized to the dose.

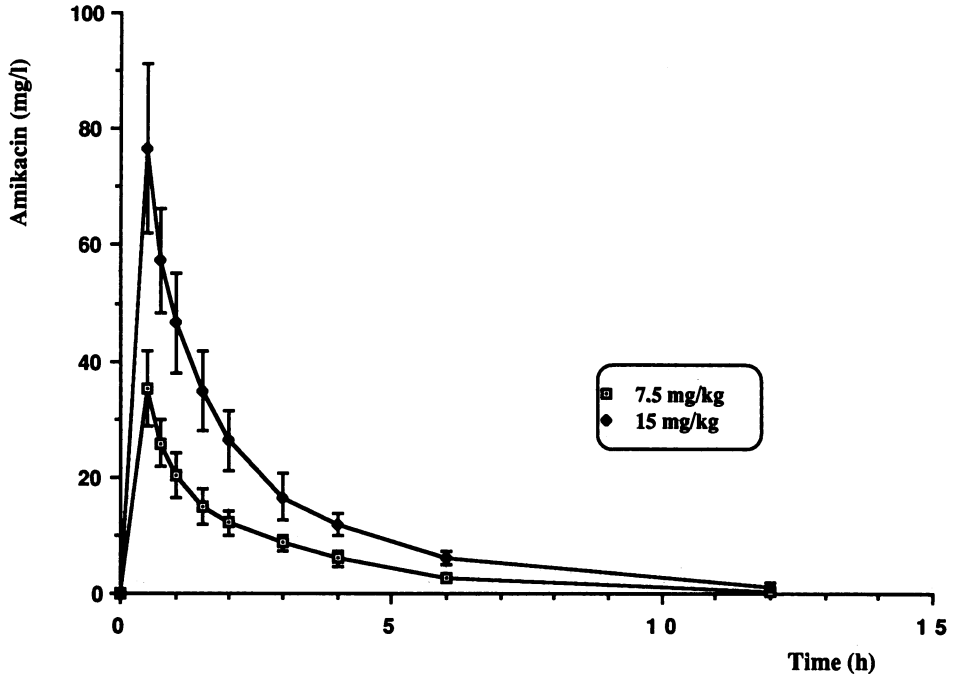

FIG. 2. Mean pharmacokinetic profiles of amikacin in serum of six healthy volunteers after administration of 7.5- and 15-mg/kg doses.

with the lowest dose. In both cases, amikacin was still present in the serum after 12 h, but at a threefold higher concentration after the 15-mg/kg dose compared with that after the 7.5-mg/kg dose. No antibiotic was found at hour 24, whatever the dose. Comparison of the elimination parameters did not reveal any significant differences, although the mean elimination half life at the β phase appeared to be slightly longer with the higher dose. The AUC obtained for the 15-mg/kg dose was 15% higher than twice the value obtained with the 7.5-mg/kg dose.

Bacteriological analysis. Figures 3 through 6 show the AUCs of the percentage of surviving bacteria expressed as a function of the amikacin concentrations in serum and their corresponding regression lines for the four selected bacteria during the first 3 and 5 h. It appears from these regression lines that after reaching a threshold of the antibiotic concentration, the bactericidal activity started and the percentage of surviving bacteria (expressed as AUC) fell rapidly as the amikacin concentration increased.

In order to estimate more precisely the parameters of the bactericidal effect, we started the regression line at the first amikacin concentration that exhibited a significant bactericidal activity (90% of the AUC). The starting point of the regression line gave the LEC, and the point where the regression lines intercepted the amikacin concentration in serum axis indicated the theoretical MEC (0% surviving bacteria). The four regression lines that were fitted under these conditions were linear and indicated that the bactericidal activity of amikacin was primarily concentration dependent. However, it was clear that the slopes obtained with *Serratia marcescens* and *Pseudomonas aeruginosa* were significantly smaller than those obtained with *Escherichia coli* or *Enterobacter cloacae*. Moreover, for the two first strains there was a significant difference ($P < 0.001$) between the slopes that were observed after 3 and 5 h. This is likely a consequence of a relatively limited concentration-dependent amikacin bactericidal activity against these two species, and a prolonged time of contact between antibiotic and bacteria may improve the in vivo bactericidal activity. Indeed, MEC during the early phase, after 3 to 5 h of contact, indicated that the greatest differences between amikacin concentrations needed to kill all the bacteria were observed with *Pseudomonas aeruginosa* (3,460 μg/ml) and *Serratia marcescens* (615 μg/ml), while *Escherichia coli* (54 μg/ml) and *Enterobacter cloacae* (39 μg/ml) gave the lowest values. Differences in LECs were less significant, but the increase in contact time resulted in a slight decrease in the concentration of amikacin required to be effective (Table 2). Moreover, in each case there was a threshold concentration under which complete bacterial regrowth occurred. This parameter, MCR, gave values of 12, 10, 9, and 16 mg/liter for *Escherichia coli*, *Enterobacter cloacae*, *Serratia marcescens*, and *Pseudomonas aeruginosa*, respectively.

DISCUSSION

The methods generally proposed for determination of the theoretically optimal dosage to be evaluated during the clinical development of a new antibiotic are essentially based on classical parameters, i.e., determination of the spectrum of antibacterial activity (MIC, MBC, or both), safety in animal models and humans, and pharmacokinetics. Although these parameters are fundamental and provide clinicians with important information, they have their limits. The laboratory assay determines MICs and MBCs in a static situation in which the antibiotic concentration remains the same over the whole incubation period. However, the situation in vivo is dynamic, with both concentration and host factors being in a state of flux. Moreover, the bacterial inoculum is largely higher in in vivo than it is in in vitro MIC experiments. Therefore, it is unclear to what extent in vitro MICs or MBCs correlate with their values in vivo. This may explain why these methods are poor predictors of clinical efficacy. For example, clinical failures have been reported with antibiotics, even though activity against the infective

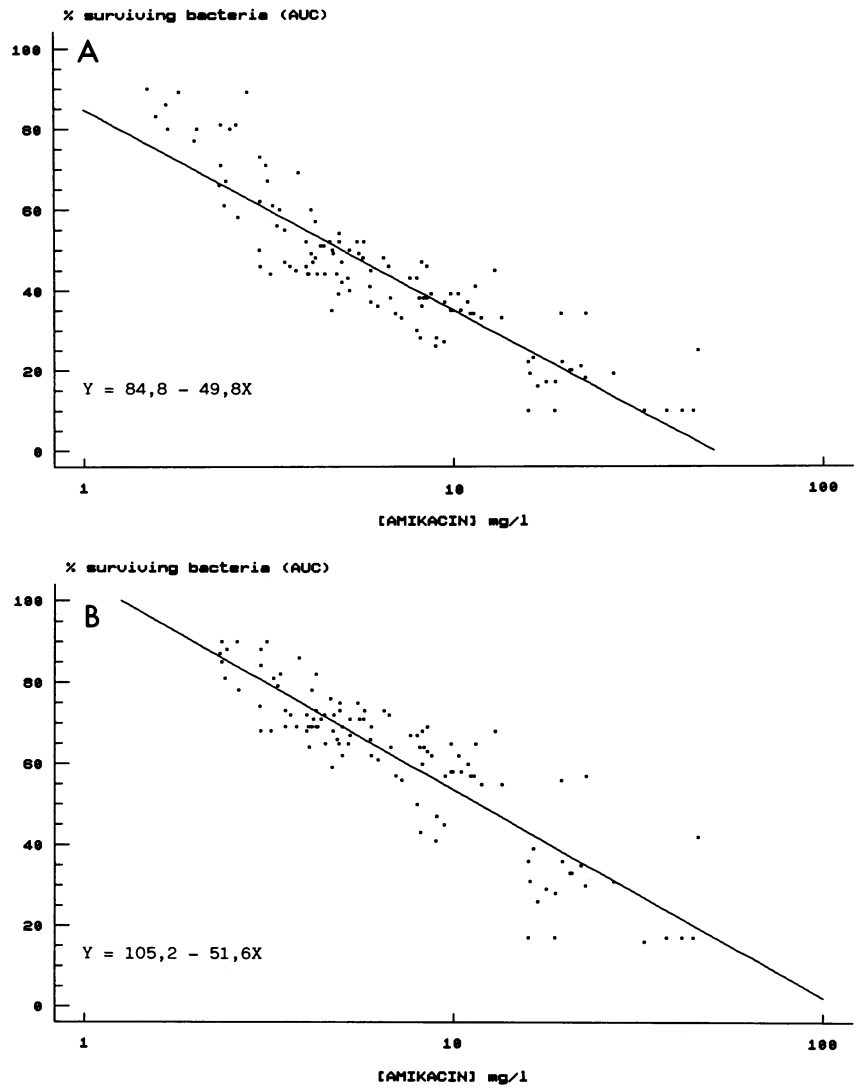

FIG. 3. Relationship between ISB and amikacin concentrations for *Escherichia coli* after 3 (B) and 5 (A) h of contact.

bacteria was confirmed in vitro (11). In animal models of experimental endocarditis it has been proved that MICs and MBCs, bactericidal activity in serum, and antibiotic concentrations are unsatisfactory predictors of in vivo efficacy (T. Calandra, P. Meyland, J. Entenza, P. Francioli, and M. P. Glauser, 25th ICAAC, abstr. no. 469, 1985; G. Potel, B. Pangon, B. Fantin, J. M. Vallois, F. Faurisson, and C. Carbon, 26th ICAAC, abstr. no. 575, 1986). However, Mouton et al. (9) found a good correlation between the bacterial resistance determined by the MIC and clinical failures. So, the MIC could be considered as a good parameter for determination of bacterial resistance but inadequate for determination of antibiotic efficacy against susceptible bacteria. On the other hand, pharmacokinetic data are usually determined without searching for any relation of those data to the antibiotic killing rate, which is still poorly documented and may also be responsible for clinical failures. Thus, the search for pharmacodynamic parameters of antibiotic activity that can be related to individual pharmacokinetic data remains a major tool.

In recent years, an additional dimension of antibacterial activity has been found in the killing rate of antibiotics, as determined from time-kill curves and regrowth patterns (8). In the present study, we aimed to combine in vivo pharmacokinetic profiles with killing curves determined ex vivo by using the serum of healthy volunteers tested against several bacterial strains. The purpose of this study was to propose a new method that links pharmacokinetic and pharmacodynamic parameters for determination of the optimal dosage regimen, as described by Schentag et al. (10), who suggested that killing curves could be a good pharmacodynamic parameter. Vogelman and Craig (13) have introduced the kinetics of antimicrobial activity, providing a new concept for the selection of an antimicrobial dosing regimen in future preclinical and human trials. More recently, they have confirmed (14), in the neutropenic mouse thigh model, the concentration or time dependence of the bactericidal activities of several antibiotics. They demonstrated that factors such as the length of time the antibiotic concentrations exceed the MIC, the postantibiotic effect, and the concentration-dependent killing can be used together to take a decision regarding the optimum antibiotic dosing regimen in a particular setting.

However, studies in humans remain uncommon. In this

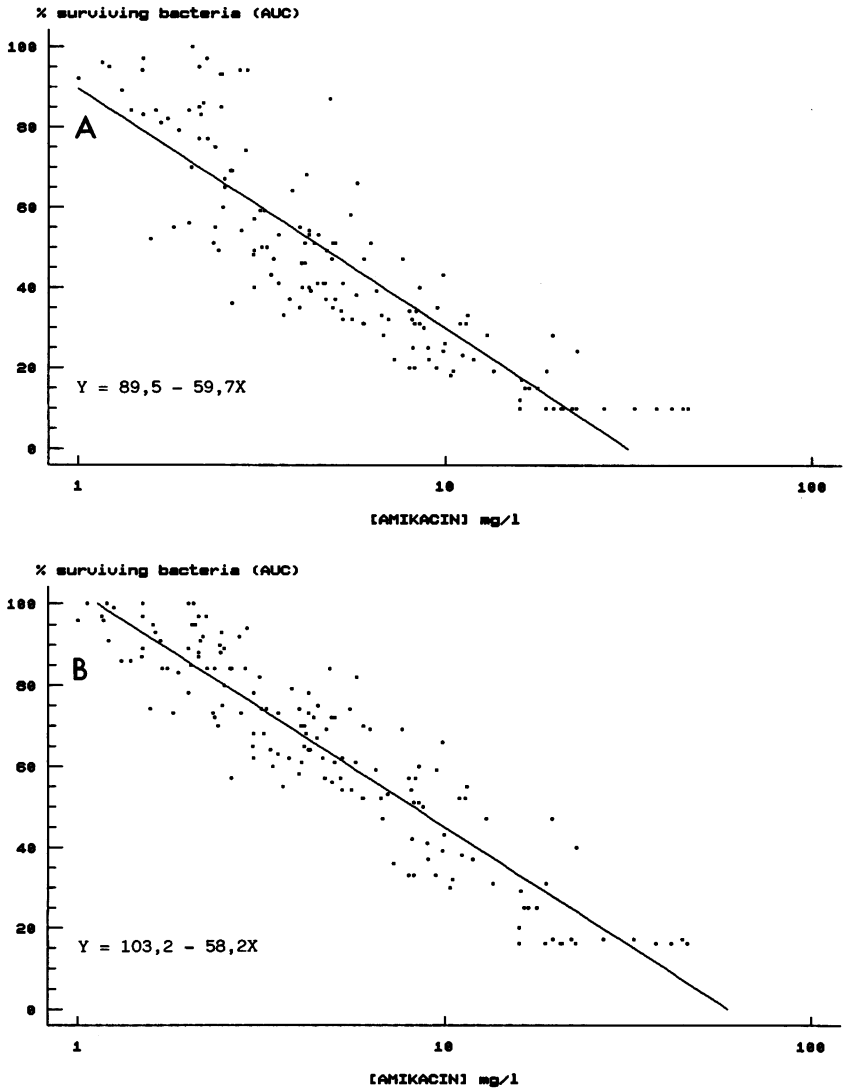

FIG. 4. Relationship between ISB and amikacin concentrations for *Enterobacter cloacae* after 3 (B) and 5 (A) h of contact.

field, Van der Auwera et al. (12) have shown that the killing rate of serum determined in volunteers is a reliable method of comparing dosing regimens.

Our study was designed to investigate the bactericidal activity of two amikacin doses in volunteers. We defined various new parameters in order to quantify the concentration-effect relationship. The ISB is a major pharmacodynamic parameter, and its expression is not very different from the killing index proposed by Bergeron and Turcotte (1), which was calculated by dividing the AUC of the number of bacteria killed versus time by the number of bacteria at the beginning and during the time course of therapy. This mathematical approach presents the advantage of evaluating the overall bactericidal activity over a determined period of time. The calculated MECs and LECs, when related to in vivo concentrations, can be used to estimate the ability of a determined dosage to reach complete or partial activity. The value of the slope obtained by correlating the ISB and the in vivo concentration of amikacin indicated the concentration or time dependence of the bactericidal activity: the more negative the slope, the faster the rate of killing and the greater concentration dependence of the bactericidal activity.

In our experiments, the pharmacokinetic behavior of the two amikacin test doses was not dose related. After a 7.5-mg/kg dose, amikacin concentrations in serum in volunteers during the first hour after administration varied from 20 to 30 mg/liter. When these values were related to killing curves, it appeared that they provided substantial but submaximal bactericidal activity against *Escherichia coli* and *Enterobacter cloacae*. When the dose administered was 15 mg/kg, the concentrations in serum reached values ranging from 45 to 77 mg/liter, which may be fully bactericidal for these strains. Moreover, the amikacin concentration remained in excess of the MCR for these two strains as long as 3 h after the 7.5-mg/kg dose and 6 h after the 15-mg/kg dose. This should be related to the concentration dependency of the amikacin bactericidal activity against these strains and supports the statement of Vogelman and Craig (13) that clinical improvement may be increased by aminoglycosides at larger doses even if they are given at less frequent intervals. The results obtained for *Serratia marcescens* and *Pseudomonas aeruginosa* were somewhat different. Indeed, at the third hour after administration the mean amikacin level in volunteers who received the 7.5-mg/kg dose was close to 9 mg/liter, which resulted in an ISB of 75% for

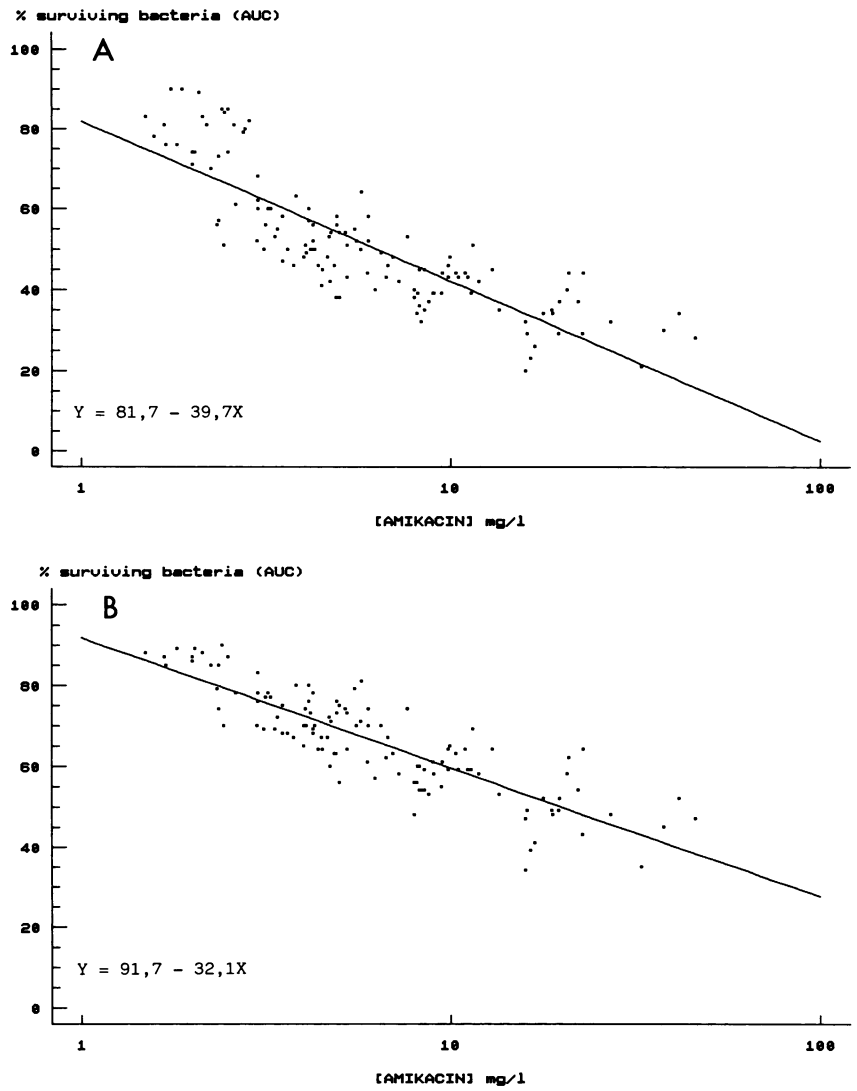

FIG. 5. Relationship between ISB and amikacin concentrations for *Serratia marcescens* after 3 (B) and 5 (A) h of contact.

Pseudomonas aeruginosa and 60% for *Serratia marcescens*. This amikacin level is very similar to the MCR for *Serratia marcescens* but is substantially below MCR for *Pseudomonas aeruginosa*. When 15 mg/kg was infused, the mean amikacin level was about 17 mg/liter and the bactericidal activity increased slightly, and the ISBs were 70 and 50%, respectively, for the two strains. At the fifth hour, after a 7.5-mg/kg dose, the ISB remained unchanged for *Pseudomonas aeruginosa* and decreased to 50% for *Serratia marcescens*, whereas the corresponding ISB values after a 15-mg/kg dose were 70 and 40%. Thus, the length of time over which high amikacin levels are maintained was also an important parameter of the bactericidal activity against these strains. At this dose, the concentration dependence was not the exclusive and sufficient parameter of efficacy.

With the exception of *Pseudomonas aeruginosa*, the MCRs determined by our methodology for the tested strains did not differ greatly, and the highest ratio was 1.8. This should be compared with the MICs, for which the ratio between the organisms ranged from 1 to 4. The MCR is an important parameter for the determination of the susceptibility of more resistant bacteria that are not detected individually during the MIC calculation. One would expect that under in vivo conditions the bacterial inoculum would be larger and that the more resistant bacteria would become dominant after the first antibiotic administration and result in clinical failures if their resistance levels were too high, thus necessitating the use of another effective companion antibiotic. Moreover, it has been shown recently (6) that the amikacin and tobramycin killing rates are slower than the gentamicin killing rate, with there being a more marked effect of time of contact between aminoglycoside and bacteria.

The values obtained in our experiments were generally below the mean amikacin levels in volunteers. However, when in vivo antibiotic levels were close to or lower than the MCR, a combination with another antibiotic is needed. Our results provide important information and may offer guidelines for clinical applications. On the basis of the pharmacokinetics in humans and the bactericidal activity of amikacin ex vivo against *Escherichia coli* and *Enterobacter cloacae*, it appears that the higher, 15-mg/kg, dose could be used with an interval of 24 h between consecutive infusions, i.e., as a once a day dosage regimen. In contrast, with *Pseudomonas*

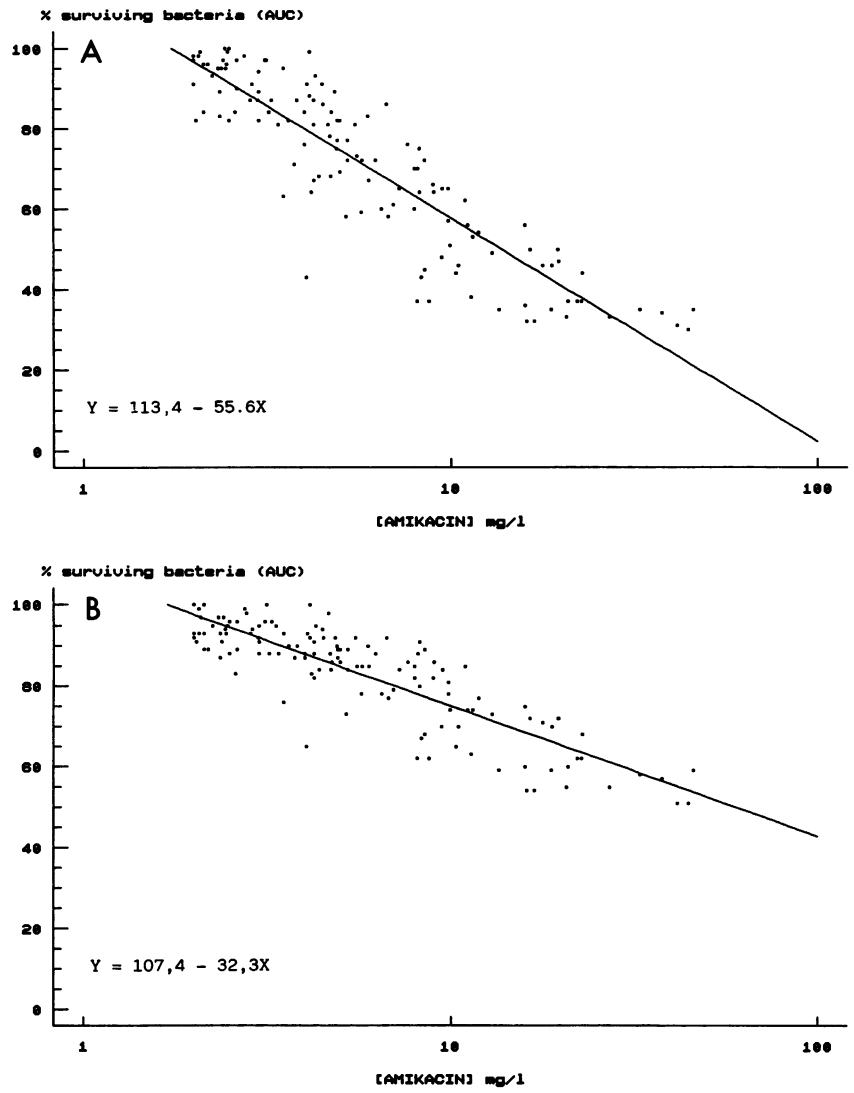

FIG. 6. Relationship between ISB and amikacin concentrations for *Pseudomonas aeruginosa* after 3 (B) and 5 (A) h of contact.

aeruginosa and probably with *Serratia marcescens*, the bactericidal effect of amikacin would not be effective for a 24-h period because of the partially time dependent activity, even including the previously described postantibiotic effect of 2 to 3 h. Thus, it would be necessary to determine another dosage regimen (two or three times a day) or to use another companion antibiotic in order to obtain sufficient efficacy through bactericidal synergy. The response can only be determined by clinical trials. These data are in agreement with those noted by Vogelman et al. (14) in animal models, which indicated a reduced efficacy of tobramycin against *Pseudomonas aeruginosa* or gentamicin against *Escherichia coli* when the dosage intervals were increased.

TABLE 2. Slopes of regression lines and MECs and LECs after 3 and 5 h of contact between amikacin and the four bacterial strains

Strain	Slope of the regression line at:		Statistical significance	MEC (μg/ml) at:		LEC (μg/ml) at:	
	3 h	5 h		3 h	5 h	3 h	5 h
Escherichia coli	−52.6	−49.76	NS[a]	115	51	2	0.6
Enterobacter cloacae	−58.23	−59.68	NS	75	36	1.4	0.7
Serratia marcescens	−32.17	−38.36	$P < 0.001$	740	125	1.1	0.4
Pseudomonas aeruginosa	−32.27	−55.6	$P < 0.001$	3,600	140	2.7	1.4

[a] NS, Not significant.

In conclusion, our study demonstrates that in a human in vivo and ex vivo model the dose-response relationship combined with the pharmacokinetics of amikacin differ, depending on the type of organism. This model presents the advantage that it was performed in humans and provides information that is directly applicable to patients. It would be relevant to determine a theoretical dosage regimen for comparative trials or to propose newer methods of evaluating investigational and already marketed antimicrobial agents. The proposed model provides valuable information but also highlights the need to confirm results obtained in animal models by clinical trials in humans.

LITERATURE CITED

1. **Bergeron, M. G., and A. Turcotte.** 1986. Penetration of cefixime into fibrin clots and in vivo efficacy against *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Antimicrob. Agents Chemother. **30**:913–916.
2. **Decazes, J. M., J. D. Ernst, and M. A. Sande.** 1983. Correlation of in vitro time-kill curves and kinetics of bacterial killing in cerebrospinal fluid during ceftriaxone therapy of experimental *Escherichia coli* meningitis. Antimicrob. Agents Chemother. **24**:463–467.
3. **Drake, T. A., C. J. Hackbarth, and M. A. Sande.** 1983. Value of serum tests in combined drug therapy of endocarditis. Antimicrob. Agents Chemother. **24**:653–657.
4. **Gerber, A. V., and C. Feller-Segesenmann.** 1986. In vivo assessment of in vitro killing patterns of *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **15**(Suppl. A):201–206.
5. **Iliadis, A.** 1985. Apis: a computer program for clinical pharmacokinetics. J. Pharm. Clin. **4**:573–577.
6. **Juvin, M. E., H. B. Drugeon, J. Caillon, and J. L. Pirault.** 1987. Comparaison de l'activité bactéricide de 3 aminosides: gentamicine, tobramycine, amikacine. Pathol. Biol. **35**:461–465.
7. **Klasterky, J., D. Daneau, and G. Swing.** 1974. Antibacterial activity in serum and urines as a therapeutic guide in bacterial infections. J. Infect. Dis. **129**:187–193.
8. **Krogstadt, D. J., and R. C. Moellering.** 1986. Antimicrobial combinations, p. 537–595. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. The William & Wilkins Co., Baltimore.
9. **Mouton, Y., C. Beuscard, O. Leroy, M. Pappo, H. B. Drugeon, et Groupe d'Étude.** 1988. Etude prospective randomisée controlées de l'association ceftazidime pefloxacine versus ceftazidime amikacine dans le traitement empirique de pneumonies et septicemies nosocomiales de réanimation: résultats préliminaires. Presse Med. **17**:1928–1932.
10. **Schentag, J. J., D. J. Swanson, and I. L. Smith.** 1985. Dual individualization: antibiotic dosage calculation from the integration of "in vitro" pharmacodynamics and "in vivo" pharmacokinetics. J. Antimicrob. Chemother. **15**(Suppl. A):47–57.
11. **Staley, T. A., and J. Bragonje.** 1976. Resistance to nalidixic acid, misconception due to underdosage. J. Am. Med. Assoc. **236**:1857–1860.
12. **Van der Auwera, P., J. Klastersky, S. Lieppe, M. Husson, D. Lauzon, and A. Pascual Lopez.** 1986. Bactericidal activity and killing rate of serum from volunteers receiving pefloxacin alone or in combination with amikacin. Antimicrob. Agents Chemother. **29**:230–234.
13. **Vogelman, B. S., and W. A. Craig.** 1986. Kinetics of antimicrobial activity. J. Pediatr. **108**:835–840.
14. **Vogelman, B. S., S. Gudmunsdsson, J. Legett, J. Turnidge, S. Ebert, and W. A. Craig.** 1988. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in animal models. J. Infect. Dis. **158**:831–847.
15. **Zak, O., and M. A. Sande.** 1983. Correlation of "in vitro" antimicrobial activity of antibiotics with results of treatment in experimental animal models and human infection, p. 55–57. *In* L. D. Sabath (ed.), Action of antibiotics in patients. Hans Huber Publishers, Bern, Switzerland.