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# Pharmacokinetic and Pharmacodynamic Approach for Comparing Two Therapeutic Regimens Using Amikacin

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**The efficiencies of two dosage schedules of amikacin (2 × 10 mg/kg of body weight per 24 h and 1 × 20 mg/kg/24 h intramuscularly for 5 days) against *Pseudomonas aeruginosa* sepsis in rabbits were compared. Blood samples were drawn at various times after the first application, and amikacin concentrations in serum were assayed microbiologically. The dynamics of the bactericidal effect of amikacin was simulated in vitro with the same strain of *P. aeruginosa*. No regrowth was found with the 20-mg/kg dose when the bacterial inoculum was in contact with experimental and theoretically predicted serum amikacin concentrations. The killing effect was present even when the drug levels decreased considerably below the MIC. The interrelationship between simulated amikacin concentrations in serum and the corresponding average killing rates was described appropriately by the standard $E_{max}$ model. The higher amikacin dose performed its bactericidal effect faster and the drug persisted longer in the blood. The two amikacin regimens were therapeutically equivalent, but the once-daily schedule had some advantages over the twice-daily drug administration which became evident when both the pharmacokinetic and the pharmacodynamic parameters of the drug were considered.**

Aminoglycoside antibiotics play a major role in the therapy of severe gram-negative infections. *Pseudomonas aeruginosa* sepsis continues to have a high mortality rate (26), so therapeutic strategies to overcome this problem were investigated. Aminoglycoside antibiotics are potent antipseudomonal agents having rapid and concentration-dependent bactericidal activity, but because of their narrow safety margins new approaches are necessary in order to optimize their therapeutic efficacy. One such approach is reducing the frequency of aminoglycoside administration (25). The pharmacodynamic background for once-daily dosing of amikacin includes concentration-dependent bactericidal activity (24), postantibiotic effect (5, 6), and low incidence of resistant subpopulations when concentrations in plasma above the threshold (8 to 10 times the MIC) are achieved (1, 21). An optimal regimen for aminoglycoside therapy would assure an adequate initial drug level, thus minimizing the opportunity for adaptive resistance, reducing the need for drug monitoring, and avoiding severe drug toxicity (18). A wide variety of animal models has been investigated, and the findings suggest that the less frequent dosing does not alter drug effectiveness (27, 34). The efficiencies of two dosage schedules of amikacin (2 × 10 mg/kg of body weight per 24 h and 1 × 20 mg/kg/24 h given intramuscularly) against experimental *P. aeruginosa* sepsis in rabbits were compared. An attempt to correlate pharmacokinetic and pharmacodynamic features of the drug simulating its bactericidal in vitro activity was made.

## MATERIALS AND METHODS

**Animals.** Chinchilla rabbits divided into three groups (six animals each) with a mean body weight of 3.12 ± 0.36 kg were included in the study.

***Pseudomonas* infection model.** Sepsis was induced by clinically isolated *P. aeruginosa* 2 (Laboratory of Clinical Microbiology, Alexander University Hospital, Sofia, Bulgaria). The MIC of amikacin for that strain was 1.5 μg/ml. *P. aeruginosa* was grown overnight on Mueller-Hinton agar, and the suspension was adjusted to give a final density of $10^9$ CFU/ml in saline. A 0.5-ml volume per kg of body weight of that suspension was injected into the marginal auricular vein. The frequency of mutation to resistance of this strain was $5.15 \times 10^{-6}$ CFU/ml when an inoculum of $10^{7-9}$ CFU/ml was used. Blood cultures were drawn before inoculation and at 1, 3, 6, 24, 48, 72, and 96 h after inoculation.

**Antibiotic and drug dosing design.** The activity of the amikacin (Pharmachim) was 710 μg/mg. The amikacin was in solution (Pharmachim ampoules, 500 mg). Amikacin was administered to rabbits according to the following schemes: 2 × 10 mg/kg/24 h and 1 × 20 mg/kg/24 h intramuscularly. The animals were treated for 5 days.

**Pharmacokinetic design and pharmacokinetic analysis.** Blood samples were drawn from the marginal auricular vein at 0.0, 0.5, 1, 2, 3, 6, and 8 h after the first administration of the drug, and the serum samples were stored at −20°C. Amikacin concentrations in serum were assayed microbiologically within 48 h by agar well diffusion method (using medium 1 according to USP XX, square plates [28 by 28 cm] with 49 wells, and *Bacillus subtilis* ATCC 3399). The agar was inoculated with a suspension adjusted by an optic standard to a density of approximately $10^9$ CFU/ml. The final inoculum was $2 \times 10^7$ CFU/ml. Each assay was performed in triplicate. In preliminary in vitro studies, antibiotic standards were prepared in a pH 7.4 buffer and in serum, and no difference between the sizes of zones of inhibition induced by a single antibiotic concentration was found. The standards (ranging from 1 to 20 μg/ml in pH 7.4 buffer) and sera were assayed without dilution. The plates were read after incubation at 37°C for 18 h. The detection limit of the microbiological method was 1 μg/ml, and intra- and interday variations were below 5%.

Pharmacokinetic parameters of amikacin in each animal were estimated by unweighted nonlinear regression analysis

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based on the Gauss-Newton computing algorithm programmed in GW-BASIC for IBM-compatible personal computers. This method incorporates linearizing of the relevant model function by a first-order Taylor series expansion in the area of initial parameters followed by multiple linear regression analysis of the data, whereby a set of improving corrections to the initial parameters are obtained at each iteration. If the sum of squares value has not been reduced after a Gauss-Newton iteration, a reduction factor is required. This efficiently reduces (damps) oscillations in the iterative process and lowers the risk of divergence (35). Asymptotic standard deviations (SD) of the model parameters were calculated according to the formula $SD_i = S \times D_i = S \times A_{ii}$ where $SD_i$ is the asymptotic SD of the $i$th parameter, $S$ is the sum of the squared deviations of the observed and calculated values divided by the corresponding degree of freedom (number of observations minus the number of model parameters), and $A_{ii}$ is the $i$th diagonal element of the inverse to the variance-covariance matrix resulting from the Gauss-Newton algorithm. The goodness of the fit and the appropriateness of the chosen pharmacokinetic model as well as the validity of pharmacokinetic parameters were assessed statistically (2, 29).

**Pharmacokinetic and pharmacodynamic modelling.** The dynamics of the bactericidal effect of amikacin was simulated in vitro with the same strain of *P. aeruginosa* as that inducing the experimental infection. Killing curves were determined for cultures in Mueller-Hinton broth incubated in a shaking device at 37°C. An inoculum of $5 \times 10^6$ CFU/ml was exposed to the constant averages of experimental serum drug concentrations determined at the different time intervals in both amikacin-treated groups. Along with that, an inoculum of $5 \times 10^6$ CFU/ml was exposed to the in vitro-simulated experimentally determined amikacin concentrations in serum (by 6 h for the 10-mg/kg group and by 8 h for the 20-mg/kg group) and afterwards to the theoretically predicted ones. After inoculation and vortexing, samples were taken every 30 min during the first 2 h and at 3, 4, 5, 6, 12, and 24 h and then diluted appropriately and seeded onto agar plates. Colony counting was performed after incubation at 37°C for 24 h. Killing rate constants at different experimental maximum concentrations of drug in serum ($C_{max}$) were estimated by plotting log CFU per milliliter versus time. Both killing half-lives were calculated as ln2/killing rate constants. Average killing rates were evaluated by linear regression according to the following equation:

$$\text{Average killing rate (log CFU, ml}^{-1} \cdot \text{h}^{-1} = 1/n \sum \frac{(\text{log CFU/ml at } t) - (\text{log CFU/ml at } t_i)}{t_i}$$

where $t_i$ is the time when the samples (three to six samples for each $t_i$) were taken and $n$ is the number of time intervals. Analysis of the dependency of average killing rates on simulated amikacin concentrations in serum at different time intervals revealed that the relationship could be described by the standard $E_{max}$ model (19), which was in good agreement with the predictions of Holford and Sheiner (17) and with the law of parsimony on the one hand and on the other hand with the competitive nature of aminoglycoside bactericidal activity (9, 19): $E_{k(i)} = E_{k(max)} \times C_i/(EC_{50} + C_i)$ where $E_{k(i)}$ is the average killing rates at different drug concentrations (see the equation above), $E_{k(max)}$ is the maximal killing rate, $C_i$ is simulated experimentally measured drug concentrations at different time intervals, and $EC_{50}$ is the concentration at which the killing rate is at half maximum.

**Statistics.** Variables are presented as means ± SD, and the differences were assessed by the two-sided $t$ test at a 95% level of significance.

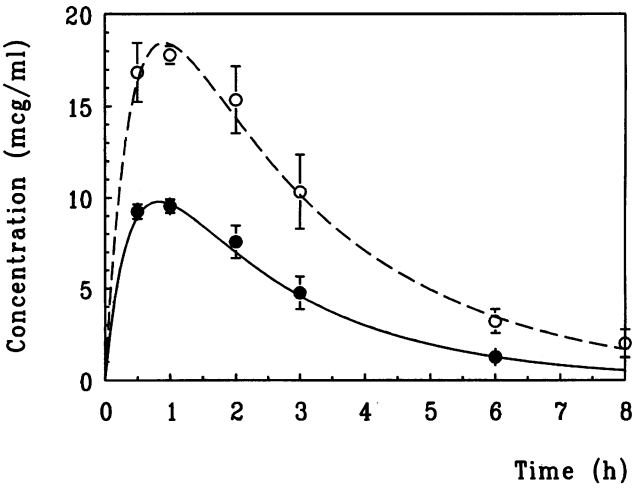


FIG. 1. Mean amikacin concentrations in serum fitted according to the one-compartment open model after intramuscular administration of the drug. Each mean represents the data from six animals ($\bar{x}$ ± SD). ●, 10 mg/kg; ○, 20 mg/kg.

## RESULTS

***Pseudomonas* infection model.** One hour after inoculation, all animals were septicemic. By 24 to 48 h after infection, the controls died. The blood cultures for all amikacin-treated animals became negative 24 h after inoculation. The treated animals survived the 15-day period of observation.

**Pharmacokinetic analysis.** The pharmacokinetic analysis revealed that the one-compartment open model after extravascular administration of the drug applied to the experimental data (coefficient of determination (CD) = 0.997 for the 10-mg/kg group and CD = 0.996 for the 20-mg/kg group) (Fig. 1). The mean values of pharmacokinetic parameters are presented in Table 1. Significant differences were found between dose-dependent pharmacokinetic parameters ($P < 0.001$) and in the elimination phase ($P < 0.05$).

**Pharmacokinetic and pharmacodynamic analysis.** The comparison of in vitro killing kinetics of the simulated serum amikacin concentrations at different time intervals revealed

TABLE 1. Pharmacokinetic features of amikacin

| Pharmacokinetic parameter[a] | $\bar{X}$ ± SD | | Statistical significance |
|---|---|---|---|
| | 10-mg/kg group | 20-mg/kg group | |
| $C_{max}^{exp}$ (μg · ml$^{-1}$) | 9.64 ± 0.39 | 17.95 ± 0.83 | $P < 0.001$ |
| AUC (μg · h · ml$^{-1}$) | 33.95 ± 3.41 | 73.09 ± 9.70 | $P < 0.001$ |
| $V$ (ml) | 2,098.5 ± 262.98 | 2,459.5 ± 484.35 | NS[b] |
| $T_{max}$ (h) | 0.84 ± 0.10 | 0.92 ± 0.096 | NS |
| CL (ml · min$^{-1}$) | 14.88 ± 1.86 | 14.66 ± 2.70 | NS |
| $t_{1/2}$ (h) | 1.64 ± 0.197 | 1.94 ± 0.24 | $P < 0.05$ |
| $k_a$ (h$^{-1}$) | 2.61 ± 0.56 | 2.48 ± 0.43 | NS |
| $k_{el}$ (h$^{-1}$) | 0.43 ± 0.049 | 0.36 ± 0.043 | $P < 0.05$ |
| $C_{max}^{th}$ (μg · ml$^{-1}$) | 10.01 ± 0.32 | 18.68 ± 1.35 | $P < 0.001$ |

[a] $C_{max}^{exp}$, experimental concentration in serum; AUC, area under the concentration-time curve; $V$, volume; $T_{max}$, time to maximum concentration in serum; CL, clearance; $t_{1/2}$, half-life; $k_a$, absorption rate constant; $k_{el}$, elimination rate constant; $C_{max}^{th}$, theoretical concentration in serum.

[b] NS, not significant.

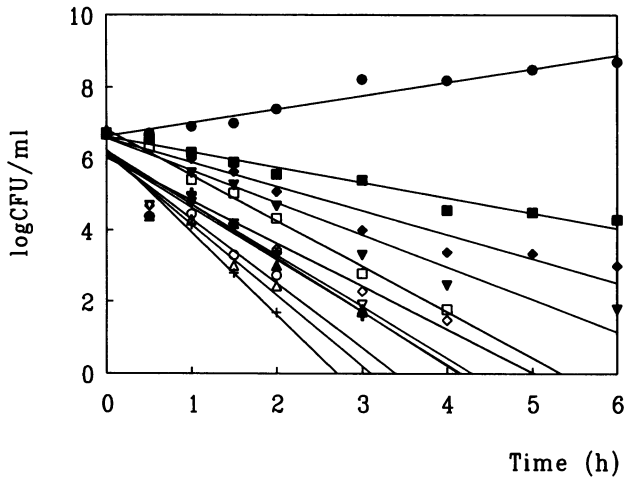


FIG. 2. Linear time dependency of amikacin killing activity on *P. aeruginosa* 2 at simulated serum drug concentrations. Amikacin was introduced at time zero at the following concentrations (in micrograms per milliliter): none (control) (●), 17.79 (+), 16.85 (△), 15.34 (○), 10.31 (▲), 9.53 (✚), 9.24 (▽), 7.56 (◇), 4.76 (□), 3.21 (▼), 2 (◇), and 1.26 (■).

concentration-dependent killing activity: the higher concentrations led to an increase in the average bactericidal rate (increase of the slope value) (Fig. 2). The analysis of the killing curves of *P. aeruginosa* 2 induced by the two highest amikacin concentrations, determined at the first hour, showed no regrowth for the once-daily dosing concentration only (Fig. 3). No regrowth was found when the bacterial inoculum was in contact with experimental and theoretically predicted serum amikacin concentrations after the higher single dose. The bactericidal effect was still present even when the drug concentrations decreased considerably below the MIC. After the sixth hour of incubation, some changes in the appearance of the colonies were observed with the higher dose in comparison with the lower one and the controls. Comparison of killing rate

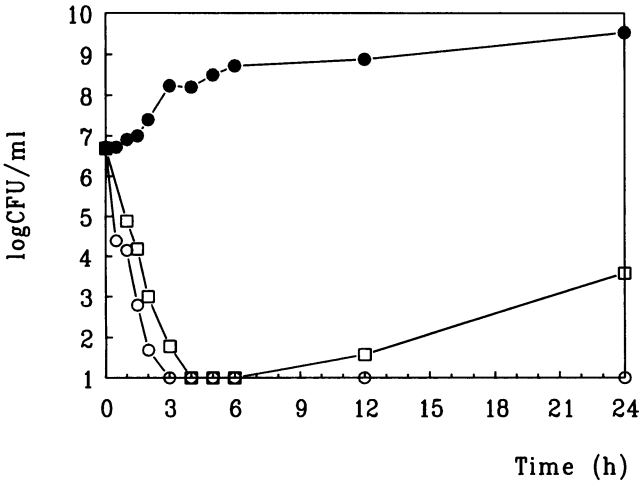


FIG. 3. Killing curves of *P. aeruginosa* 2 exposed to the highest experimental concentrations determined at the first hour after intramuscular application of amikacin. Amikacin was introduced at time zero at the following concentrations (in micrograms per milliliter): none (control) (●), 17.79 (○), and 9.53 (□).

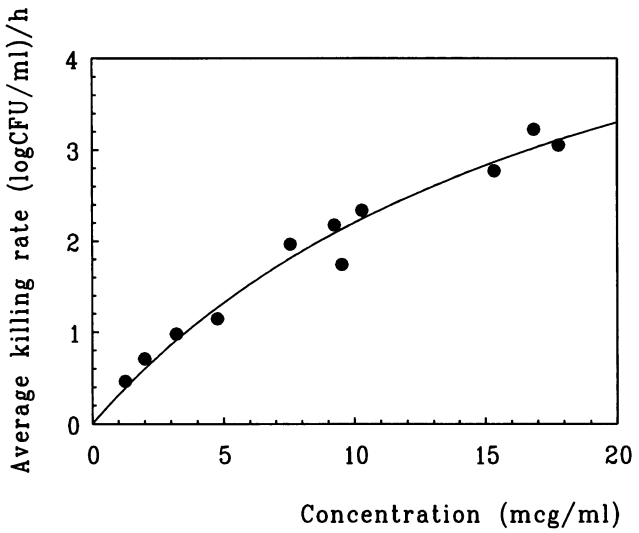


FIG. 4. Nonlinearity of amikacin killing kinetics for *P. aeruginosa* 2 in dependency of simulated serum drug concentrations.

constants (1.176 versus 2.118 $h^{-1}$) and killing half-lives (0.589 versus 0.327 h) derived from in vitro simulation of amikacin bactericidal activity at different $C_{max}$ revealed about a twofold-faster in vitro killing effect of the drug with the $C_{max}$ achieved after once-daily administration of 20 mg of amikacin per kg. The interrelationship between the simulated serum amikacin concentrations and the corresponding average killing rates was described appropriately by the $E_{max}$ model (CD = 0.968) (Fig. 4). The parameters of the $E_{max}$ model are as follows. For the $E_{k(max)}$ (log CFU per milliliter per hour), $\bar{x}$ ± SD was 6.58 ± 1.164 ($t$ = 18.757). For the $EC_{50}$ (micrograms per milliliter), $\bar{x}$ ± SD was 19.90 ± 5.678 ($t$ = 11.622).

## DISCUSSION

Appropriate dosing schedules are an important factor for successful antibiotic therapy. Available data on aminoglycoside dosage regimens reveal that once-daily administration is not a new issue but has been discussed for more than 15 years (21). The efficacies of continuous infusion or frequent dosing and infrequent (as little as once-daily) administration of a single amount of aminoglycoside were compared in a variety of animal studies (13–15, 20). Since amikacin is known to be a concentration-dependent antimicrobial drug (1, 24, 31), the established significant differences in the dose-dependent pharmacokinetic parameters support the statement for therapeutic advantages of the high-dose schedule in comparison with the low-dose schedule. Aminoglycoside antibacterial efficacy in animal models is greater with treatment regimens that result in the highest peak concentrations in serum and the largest areas under the time-concentration curve (10).

Usually, pharmacokinetic and pharmacodynamic evaluations are performed independently without a search for an interrelationship (11). The determination of the killing curves and the killing rates allows the introduction of an efficiency parameter (12). Coupling of in vitro killing kinetics (instead of the traditional MIC) with drug pharmacokinetic profile more closely simulates in vivo conditions (28).

The introduction of pharmacodynamic parameters such as killing rate constant and killing half-life facilitates the integration of pharmacokinetic and pharmacodynamics data. Thus it is found that the higher amikacin dose causes bactericidal

activity almost twice as fast as the lower dose. This is in good agreement with the previous statement (26) for a high initial peak concentration associated with prolonged washout before re-exposure. The analysis of the killing curves confirms the biphasic bactericidal action of amikacin. According to MacArthur et al. (22) and Jackson et al. (18), the primary bactericidal phase is rapid and drug concentration dependent. During this initial phase, the killing rate is directly related to the initial drug concentration. The second phase is independent of the drug concentration, and the bactericidal rate is low. A fact of interest is the continuous bacterial killing observed after decreasing of the drug concentration considerably below the MIC (1.5 μg/ml). The efficacy of aminoglycoside correlates with the magnitude of the inside-negative transmembrane potential, which serves as a driving force for the internalization of the polycationic aminoglycosides (3). Hancock and Bellido (16) consider that a critical level of aminoglycosides must be achieved intracellularly in order to trigger irreversible metabolic disturbances. This critical intracellular level could be achieved only if a high antibiotic concentration is available in the environment (medium or serum). After exposure to a concentration of 17.79 μg/ml, the bacteria probably underwent some changes which made their death thereafter inevitable regardless of the available drug concentration. The appearance of the modified *P. aeruginosa* 2 colonies may be due to amikacin-selected small-colony resistant mutants which had respiratory transport enzyme changes that resulted in a marked reduction of the transmembrane potential (3, 4).

By using the $E_{max}$ model, the amikacin bactericidal action against *P. aeruginosa* 2 was quantified, expressed by the kinetic parameter $EC_{50}$ (19.90 μg/ml). The value for $E_{k(max)}$ (6.58 CFU $ml^{-1}$ $h^{-1}$) approximates the value of the initial inoculum and is an estimation of intrinsic killing activity of the drug. An advantage of such an integrated approach is the possibility of predicting amikacin killing activity in the high- and low-concentration ranges. The model assumes first-order kinetics after low doses and zero-order kinetics after high doses. The biological meaning of these results is that with momentary contact with the drug at a concentration of 19.90 μg/ml, this bacterial population should be completely killed. Because this concentration ensures the necessary bactericidal effect and its estimation is quite near to the mean $C_{max}$ after intramuscular dosing of 20 mg/kg (Table 1), one could consider that no increase of the single dose is needed. At the same time, the $C_{max}$ for all animals treated once daily with the high amikacin dose were far below the toxic range.

From experiments on animals, Gerber et al. (13) argue against once-daily therapy with aminoglycosides in neutropenic animals with *P. aeruginosa* thigh muscle infection. Levels of antimicrobial agents in muscle interstitial fluid closely followed concentrations in serum because aminoglycosides do not penetrate into animal cells (15, 32, 33). Residual drug in thigh tissue did not cause postantibiotic effect (PAE), but levels in serum accurately predict antimicrobial activity in mouse thighs (13). In agreement with the findings of Vogelman et al. (33), it was considered that the presence of PAE in vivo is generally consistent with in vitro data. We have eliminated most of the effects of host defense by using an in vitro model simulating drug concentrations. The PAE of aminoglycosides is key in determining the optimal dosing interval for these drugs. The duration of PAE increases with higher drug concentrations, up to a maximal response. Bactericidal rates are lower in vivo than in vitro, and higher drug concentrations could be used for inducing longer in vivo PAE (26).

Daikos et al. (7, 8) investigated the kinetics of unstable first-exposure resistance and suggest that bacteria surviving after the high concentration may not begin to metabolize normally for up to 8 h after all the extracellular aminoglycoside has been washed away. Exposure of surviving bacteria to a second dose of aminoglycoside before they have recovered from the first dose seriously impairs the bactericidal effect of the second dose.

Aminoglycoside toxicity is related to the duration of drug treatment (23). Transient exposure to high aminoglycoside concentrations in serum after once-daily dosing and a long washout period with peak and trough concentrations result in less accumulation in the body fluids and tissues (23, 30). The rapid achievement of effective peak concentrations may limit the duration of exposure to the aminoglycoside and therefore may decrease the toxicity potential. Once-daily dosing and the choice of the most appropriate time of the day (middark) may help to achieve rational chronotherapeutics resulting in reduced drug toxicity (36).

The results of this study support some of the advantages of once-daily amikacin therapy (26), when drug pharmacokinetic behavior is integrated with its in vitro bactericidal activity.

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