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In Vivo Antibacterial Effects of Simulated Human Serum Profiles of Once-Daily Versus Thrice-Daily Dosing of Amikacin in a *Serratia marcescens* Endocarditis Experimental Model

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Once-daily dosage of aminoglycosides is currently under consideration. The lower toxicity of this regimen has been clearly established, but there are conflicting experimental and clinical data concerning its efficacy. It is inadvisable to optimize human therapy by extrapolation from experimental studies since animal and human pharmacokinetics differ. The simulation of human pharmacokinetics in experimental infectious models would seem to offer a more rational approach. We used computer-controlled infusion of amikacin at a variable flow rate to simulate human pharmacokinetics in a *Serratia marcescens* rabbit endocarditis model and to compare two therapeutic regimens (once-daily versus thrice-daily doses). The doses corresponded to simulations of 15 and 30 mg/kg of body weight per day in humans, and antibacterial activity was measured in vegetations (Veg) after 24 h of treatment. The results show that the dose corresponding to 15 mg/kg/day failed to produce a significant reduction of CFU (6.8 ± 0.9 and 6.4 ± 0.8 $\log_{10}$ CFU/g of Veg, respectively, for once-daily and thrice-daily doses versus 7.6 ± 1.0 for controls). A significant reduction was observed only for the dose corresponding to 30 mg/kg/day in humans (5.2 ± 1.5 and 5.4 ± 1.1 $\log_{10}$ CFU/g of Veg, respectively, for the two regimens). With this model, the efficacy of amikacin was similar for both regimens after 24 h of treatment simulating human pharmacokinetics.

Numerous papers in recent years have attempted to define the optimal therapeutic regimen for aminoglycosides. Although toxicity is lower with once-daily dosage (ODD) (3, 29, 33), the best efficacy for this mode of administration versus conventional thrice-daily dosage (TDD) remains to be determined.

Many authors have attempted to justify the use of ODD on the basis of the concentration-dependent antibacterial activity of aminoglycosides in vitro. Nevertheless, many other phenomena, such as the postantibiotic effect (10, 36) or adaptive resistance (8), are also influenced by the therapeutic regimen and can interfere with bactericidal activity. Thus, there is a lack of convincing evidence for the best dosage schedule. In situ pharmacokinetic data (e.g., concentrations achieved in the infectious focus) also influence the definition of the best dosage regimen. Thus, it has been suggested for endocarditis that high peaks (as expected with ODD) provide more rapidly effective concentrations within vegetations during the early phase of treatment (6), although these results are only theoretical and require experimental proof.

An approach based on clinical investigation has also been developed. Though some of these studies are favorable to ODD (14, 21), most report no differences between ODD and fractionated dosages in terms of efficacy (9, 15, 19, 25, 30, 31, 33, 34). Moreover, they suffer from numerous methodological problems: too few participants, use of a single-blind protocol, great diversity of infectious diseases in the patients studied, and therapies that often include various associated antibiotics. In addition, pharmacokinetic data are frequently given as means, thus preventing discriminative analysis of output variables (e.g., rates of cure or bacteriological eradication) as a function of individual pharmacokinetic parameters, whereas large interindividual pharmacokinetic variability during sepsis is a classic feature of human clinical studies (20).

Investigations using experimental infectious models are necessary to avoid such methodological difficulties. Nevertheless, extrapolation to human clinical situations requires considerable caution. These models benefit from the similarity of the pharmacological target (notably a bacterial agent in an infectious focus) relative to human infections, although the organisms involved are not as numerous as in clinical trials. Moreover, they differ in other respects, such as the nature of host defense systems and the pharmacokinetic specificities of the animal used for experimentation. Concerning the latter difference, the use of models closely approximating the conditions in which antibiotics perform in humans is highly advisable. To achieve this, a commonly used method is to administer a dose to the animal ensuring an area under the curve (AUC) similar to that in humans. The major drawback of this method is the generation of different serum drug concentration profiles despite identical AUCs. Moreover, extrapolation from results based exclusively on equalizing AUCs, without simulation of human kinetics, may lead to mistaken recommendations for clinical practice, as has already been noted for a rat model using beta-lactam with and without simulation (11). As the animals used for these models are small, they show higher clearances (1, 23). For an identical AUC, they exhibit much higher concentration peaks followed by much faster elimination than in humans. This experimental pattern impedes the interpretation of animal results concerning the respective impacts of time and concentration on the antibacterial effect in vivo. In the particular case of aminoglycosides, this aspect is

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TABLE 1. Amikacin doses administered to four groups of rabbits to simulate human plasma kinetics of four therapeutic regimens during a 24-h period

Portion	Dose (mg/kg)[a]			
	LODD (56)	LTDD (54)	HODD (112)	HTDD (108)
Bolus[b]	27	9	54	18
Infusion[c]	29	9	58	18

[a] Totals are given in parentheses.
[b] Single initial bolus for LODD and HODD or three boluses (at 0, 8, and 16 h from the beginning of treatment) for LTDD and HTDD.
[c] Variable-flow continuous infusion using a computer-controlled pump to obtain a serum drug profile with a 2-h elimination half-life. LODD and HODD, infusion during 24 h; LTDD and HTDD, infusion during 8 h repeated three times.

important in determining whether ODD or fractionated doses provide the best therapeutic regimen. The pragmatic approach of simulating human pharmacokinetics in the animal ensures greater reliability. Two simulation techniques are currently used. One lengthens aminoglycoside elimination by chemical induction of renal insufficiency (7, 10), whereas the other compensates for faster elimination of the antibiotic by a real-time administration of the drug that matches the difference between human and animal kinetics (12). The compensatory dose can be delivered by a computer-controlled pump with a preprogrammed variable flow rate (5, 11). A direct intravenous bolus is administered immediately before this infusion in order to obtain the initial targeted peak concentration. This method was used in our study to assess amikacin efficacy in a *Serratia marcescens* rabbit endocarditis model.

MATERIALS AND METHODS

Microorganism. The *S. marcescens* strain used (HN229) was isolated from the urine of a hospitalized patient and proved resistant to rabbit serum. The MIC and the MBC of amikacin against this strain were both 1 mg/liter.

Endocarditis. The animals were New Zealand White female rabbits (10 to 15 weeks of age; weight, 2 to 3.5 kg) housed in individual cages, with free access to food and water. Left endocarditis was induced as described elsewhere (27). Briefly, 24 h after insertion of a polyethylene catheter transfixing the aortic valve, each animal received 1 ml of a suspension containing 7.45 $\log_{10}$ CFU/ml, injected into a marginal ear vein. The inoculum was checked systematically by quantitative cultures immediately after injection.

Treatment. Rabbits were randomized into five groups as follows, and treatment was administered 48 h after inoculation: (i) no treatment (control group); (ii) simulation of a single injection of 15 mg/kg in humans (low-dose ODD group [LODD]); (iii) simulation of an injection of 5 mg/kg every 8 h in humans (low-dose TDD group [LTDD]); (iv) simulation of a single dose of 30 mg/kg in humans (high-dose ODD group [HODD]); (v) simulation of an injection of 10 mg/kg every 8 h in humans (high-dose TDD group [HTDD]).

Simulations. The protocol for amikacin administration was established on the basis of a one-compartment model in the rabbit in order to obtain a serum profile following a monoexponential decay with a 2-h serum apparent half-life, comparable to that in humans. To simulate the ODD regimen, the total amount of amikacin was administered to the rabbit in an initial bolus followed immediately by variable-flow infusion delivered over 24 h by a computer (PC i486)-controlled pump (Braun). To simulate the TDD regimen, the total amount was divided into three fractions for administration every 8 h in the form of an initial bolus followed by variable-flow infusion during 8 h according to a comparable protocol. The bolus and infusion were delivered through a catheter positioned in a marginal ear vein. Doses were determined during preliminary studies. The total amounts of amikacin administered over 24 h were 56, 54, 112, and 108 mg/kg, respectively, for the LODD, LTDD, HODD, and HTDD groups. Treatment details are provided in Table 1.

Pharmacokinetics. Serum samples for pharmacokinetic analysis were obtained from all treated animals by means of a catheter positioned in the median artery of the ear contralateral to drug infusion. For ODD-treated animals, blood samples were performed at 1, 2, 4, and 8 h. An additional sample was obtained at 16 h for the HODD group. For TDD-treated animals, samples were performed 1, 2, 4, and 8 h after the beginning of the first and third administrations. No study was done for the second administration. After centrifugation, serum amikacin concentrations were determined by fluorescence polarization immunoassay (TDK Abbott) (sensitivity limit, 0.8 mg/liter). Coefficients of variation were from 2.01 to 6.11% and from 3.26 to 7.5%, depending on the amikacin level, within and between runs, respectively (16). For the highest concentrations, a two- or fourfold dilution was done to retain the accuracy limits of the method. The different pharmacokinetic variables for each simulated bolus (first-order elimination constant, serum apparent half-life, concentration at time zero, distribution volume, and AUC) were calculated by logarithmic regression. For TDD animals, 24-h AUC was estimated as three times the mean value of the AUCs of the first and third 8-h periods. Clearance was calculated as the ratio between the total dose administered for 24 h and the 24-h AUC.

Treatment evaluation. Animals were sacrificed by a 100-mg thiopental intravenous bolus at the end of 24-h treatment. The heart was removed, and the vegetations were dissected and rapidly rinsed with sterile saline solution. The vegetations were weighed and then homogenized in 500 μl of sterile saline solution in a Thomas Teflon homogenizer. Samples (50 μl) of serial dilutions were then spread on trypticase soy agar plates by using a Spiral System (Interscience). The cultures were then grown for 24 h at 37°C, and the bacterial titer was expressed in $\log_{10}$ CFU/g of vegetation. With this method, quantities as low as 20 CFU/ml of homogenate could be detected. Animals for which the catheter tip was not positioned intraventricularly were eliminated. Two other rabbits with an amikacin level about five times that of the rest (most probably as a result of renal failure) were eliminated because of their aberrant kinetics. After exclusion of these technical failures, the numbers of rabbits were 16, 8, 6, 11, and 11, respectively, for controls, LODD, LTDD, HODD, and HTDD.

Statistics. Two analysis of variance (ANOVA) models were used for analysis of pharmacokinetic data. The first included administration mode (ODD or TDD) and dose (low or high) as independent crossed factors (with interaction) and the order number of the bolus (first or third) as a factor nested into the TDD administration mode. This model served for analysis of concentration at time zero, volume of distribution, first-order elimination rate constant, and apparent half-life in serum. The second model included only administration mode and dose as independent crossed factors and was used to study 24-h AUC and clearance. Analysis of the pharmacodynamic relation was performed on nonsterile animals by using the $\log_{10}$ of the surviving bacterial population as the variable. The ANOVA model included treatment (treatment versus control) as independent factor. The administration mode and the dose were included as factors nested into the treated group (crossed with each other and also studied for their interaction). The comparison of each treated group versus controls was performed with the Sheffe correction. The AUC-effect relation was studied by linear regression for treated animals as a whole and also for ODD and TDD groups separately. A threshold of 0.05 was considered significant for all statistical calculations. ANOVA models were evaluated by SuperAnova software (Abacus Concepts).

RESULTS

Pharmacokinetics. Serum amikacin levels for each treated group are indicated in Fig. 1 and 2. The pharmacokinetic parameters for the four treated groups are shown in Table 2. There were no significant differences between the observed pharmacokinetic parameters resulting from the first and third boluses in the TDD regimen, thereby justifying the calculation of the total 24-h AUC on the basis of the mean determined for the first and third boluses multiplied by 3. Distribution volume, calculated from the ratio between the initial dose and the initial concentration, was independent of the total administered dose but influenced by the therapeutic regimen, being higher for the ODD group than the TDD group (ANOVA $F = 21.7$; $P < 0.001$). There was also an interaction between the effects of the dose and the therapeutic regimen on volume of distribution (ANOVA $F = 9.7$; $P < 0.005$). Apparent half-life was influenced only by the dose, being longer with the higher dose (ANOVA $F = 14.9$; $P < 0.005$). Twenty-four-hour AUC was affected by the dose (ANOVA $F = 56.4$; $P < 0.001$) and not by the administration mode. There was a large variation in clearances for the four groups studied. Although this variation reflected the influence of administration mode (ODD or TDD; ANOVA $F = 4.9$; $P < 0.05$), there was also a significant interaction between the effect of the dose administered and the therapeutic regimen (ANOVA $F = 8.6$; $P < 0.01$). There were no significant differences according to the total dose used.

Pharmacodynamics. The comparison of results between treated groups and the control group is given in Table 3. ANOVA showed that the killing effect depended only on the dose administered (ANOVA $F = 10.7$; $P < 0.005$) and was

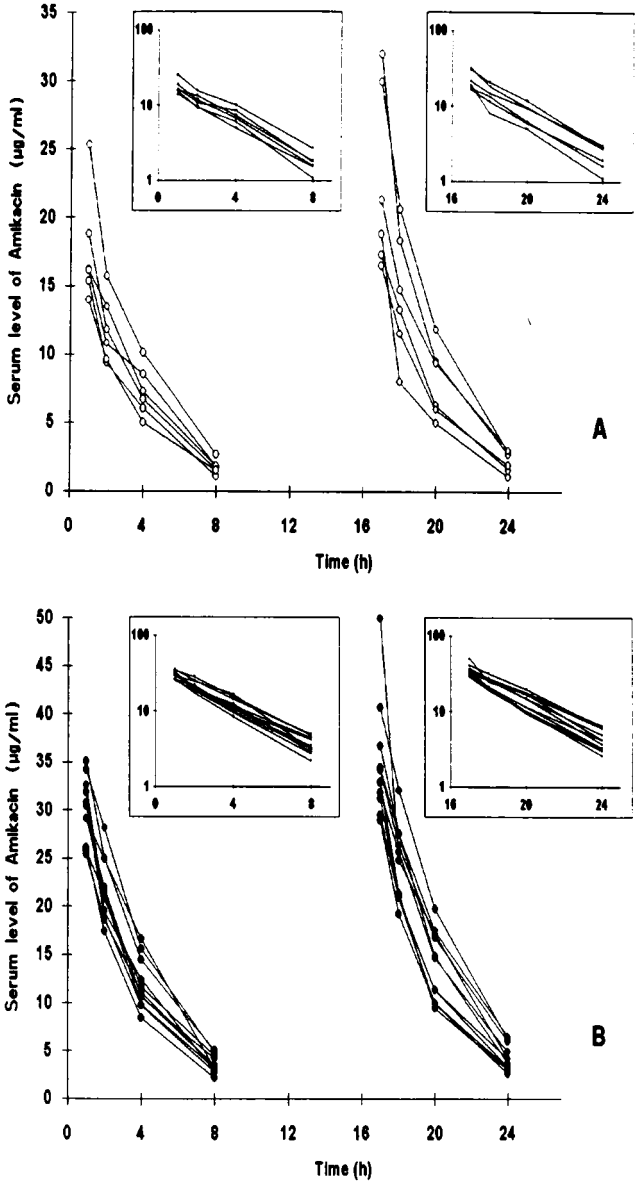

FIG. 1. Serum amikacin levels in rabbits receiving treatment simulating human pharmacokinetics of a regimen with an 8-h dosing interval during a 24-h period. Plasma assays were performed for the first and third simulated boluses. (A) Simulation of a fractional human dose of 5 mg/kg; (B) simulation of a fractional human dose of 10 mg/kg. The semilogarithmic plots are shown in the insets.

independent of administration mode. No interaction was observed between dose and administration mode. The overall correlation between total 24-h AUC and the $\log_{10}$ number of surviving bacteria at 24 h was significant ($R = 0.49$; $P < 0.01$; Fig. 3). In order to compare the two regimens, linear regression was also calculated separately for ODD and TDD. The comparison of the slopes and original ordinates for these two regressions showed no significant differences for either of the variables.

DISCUSSION

To obtain similar kinetics, the total dose given to rabbits (in milligrams per kilogram of body weight) needed to be four times as great as that given to humans. This same ratio of 4 to 1 is ordinarily used in the experimental model without simulation in order to obtain an AUC comparable to that observed in the human clinical situation (28). Doses and infusion rates in this study were based on a one-compartment model in the animal, which proved valid for simulation purposes. Monoexponential interpretation was quite suitable for the pharmacokinetics obtained, as indicated by the elevated values for the correlation coefficients corresponding to this regression. However, theoretical analysis of our simulation procedure predicted that, if there was a second quantitatively significant compartment in the animal, the resulting kinetics would have exhibited at least a second exponential decay. Moreover, no accumulation phenomenon was observed between the first and third simulated boluses in the TDD regimens.

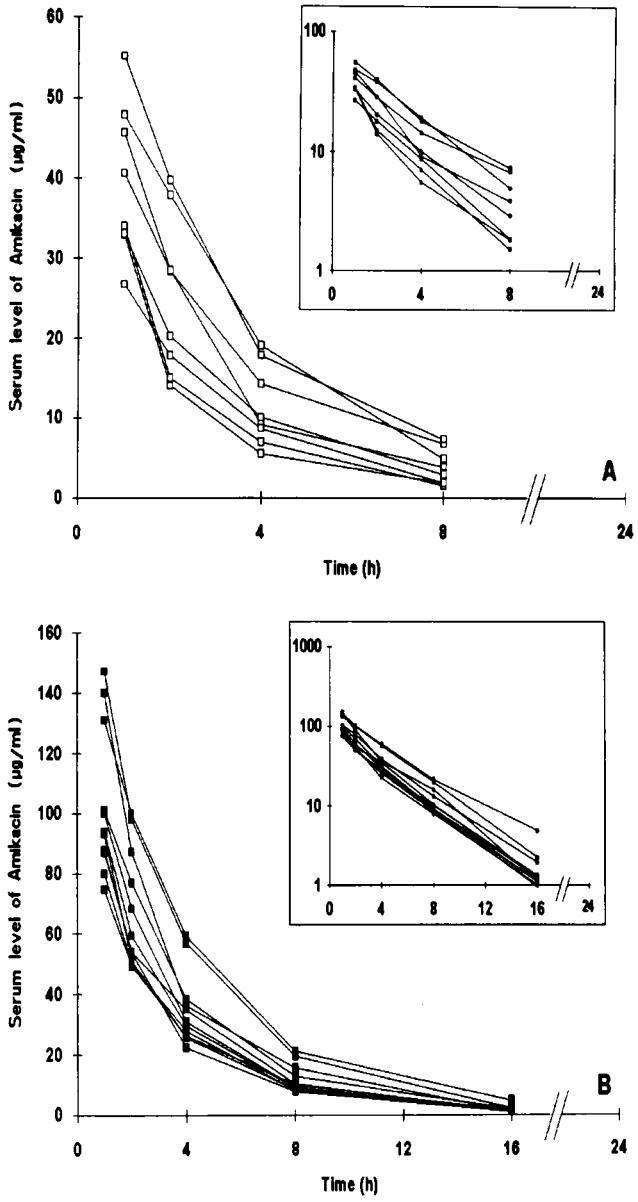

FIG. 2. Serum amikacin levels in rabbits receiving treatment simulating human pharmacokinetics of a single injection for a 24-h period. (A) Simulation of a single human dose of 15 mg/kg; (B) simulation of a single human dose of 30 mg/kg. The semilogarithmic plots are shown in the insets.

TABLE 2. Pharmacokinetics of amikacin in different therapeutic regimens[a]

Human dose[b]	Group[c] (*n*)	r^2 (range)	C_1 (μg/ml) (mean ± SD)	C_0 (μg/ml) (mean ± SD)	V (ml/kg)[d] (mean ± SD)	K_e (h^{-1})[d] (mean ± SD)	$t_{1/2}$ (h)[d] (mean ± SD)	AUC (mg · h/liter)[d] (mean ± SD)	Clearance (ml/h/kg)[e] (mean ± SD)
15	ODD (8)	0.94–0.99	39.5 ± 9.5	49.9 ± 13.1	571 ± 135	0.346 ± 0.056	2.06 ± 0.36	150.7 ± 55.5	417 ± 147
15	TDD 1 (6)	0.96–0.99	17.6 ± 4.1	24.2 ± 4.8	382 ± 71	0.327 ± 0.026	2.13 ± 0.16	69.0 ± 15.1	
15	TDD 3 (6)	0.97–0.99	22.6 ± 6.7	29.6 ± 9.0	325 ± 88	0.329 ± 0.036	2.13 ± 0.24	83.8 ± 25.2	
15	Mean (6)		20.1 ± 5.9	26.9 ± 6.3	349 ± 76	0.328 ± 0.028	2.13 ± 0.18	229.1 ± 57.4[f]	248 ± 59
30	ODD (11)	0.98–0.99	103.2 ± 24.8	118.3 ± 31.9	484 ± 113	0.278 ± 0.024	2.51 ± 0.24	429.1 ± 130.8	280 ± 70
30	TDD 1 (11)	0.96–0.99	30.0 ± 3.3	40.1 ± 4.8	454 ± 52	0.305 ± 0.028	2.29 ± 0.21	120.5 ± 16.7	
30	TDD 3 (11)	0.97–0.99	34.9 ± 6.0	45.5 ± 7.2	404 ± 57	0.299 ± 0.038	2.36 ± 0.31	139.1 ± 24.5	
30									
30	Mean (11)		32.4 ± 5.3	42.8 ± 5.7	426 ± 87	0.302 ± 0.031	2.32 ± 0.24	389.4 ± 58.6[f]	282 ± 41

[a] r^2, square of the correlation coefficient of the fit to a single exponential decay; C_1 and C_0, concentrations at 1 h and time zero, respectively; V, volume of distribution; K_e, first-order elimination constant; $t_{1/2}$, apparent half-life in serum.
[b] To simulate kinetics of the human dose of 15 mg/kg/day for the ODD and TDD regimens, doses of 56 and 54 mg/kg, respectively, were used for rabbits. To simulate a human dose of 30 mg/kg/day for ODD and TDD regimens, doses of 112 and 108 mg/kg, respectively, were used for rabbits.
[c] TDD 1 and TDD 3, first and third injections in the regimen with three injections per 24 h.
[d] Data were calculated for each animal, on the basis of the fit to a single exponential decay.
[e] Data calculated by using 24-h AUC (pooled TDD for the TDD regimen) and the total administered dose during 24 h.
[f] AUC during 24 h was estimated as $(AUC_{TDD1} + AUC_{TDD3}) \times 3/2$.

Despite these observations attesting to the validity of the model, some variability was noted. Analysis of the kinetic parameters obtained indicated a longer apparent half-life for simulations of doses corresponding to 30 mg/kg/day than those of doses corresponding to 15 mg/kg/day. Although renal function was not especially evaluated, this difference in apparent half-life may have been due to the expression of renal toxicity with the higher dose, nor can the occurrence of certain artifacts be ruled out since late samples were obtained at 16 h for the high-dose ODD group but not for the low-dose ODD group. The distribution volumes for amikacin (calculated as the ratio of the initial injected intravenous dose as a bolus to the original concentration) were higher for ODD than TDD, providing peak levels for ODD less than three times those for TDD. This result should be considered in terms of a linearity defect in aminoglycoside distribution, as previously reported for humans (30, 31, 34). An unexplained interactive effect of the administration mode and the simulated dose on volume of distribution was also noted.

In addition to these systematized variations, we observed considerable interindividual residual variability in pharmacokinetic parameters, particularly large AUC differences for the same therapeutic regimen. This variability, which was also noted for volume of distribution and apparent half-life in serum, probably reflected a corresponding variability in the animal's own pharmacokinetic parameters. Moreover, some animals showed a totally aberrant profile. Variability and aberrations of this kind were not observed during preliminary simulation studies in healthy animals (5), and it is quite likely that they resulted from septic status. In practice, particularly with models simulating human pharmacokinetics, it would seem essential to perform individual pharmacokinetic controls on the

TABLE 3. Antibacterial activity of amikacin after 24-h treatment as a function of therapeutic regimen

Human dose[a]	Group (*n*)	$\log_{10}$ CFU/g (mean ± SD)	P^b
0	Control (16)	7.6 ± 1.0	
15	ODD (7)	6.8 ± 0.9	NS
15	TDD (6)	6.4 ± 0.8	NS
30	ODD (8)	5.2 ± 1.5	<0.001
30	TDD (9)	5.4 ± 1.1	<0.001

[a] To simulate the kinetics of a human dose of 15 mg/kg/day for ODD and TDD regimens, doses of 56 and 54 mg/kg, respectively, were used for rabbits. To simulate a human dose of 30 mg/kg/day for ODD and TDD regimens, doses of 112 and 108 mg/kg, respectively, were used for rabbits.
[b] Versus the control group (Sheffe test). NS, not significant.

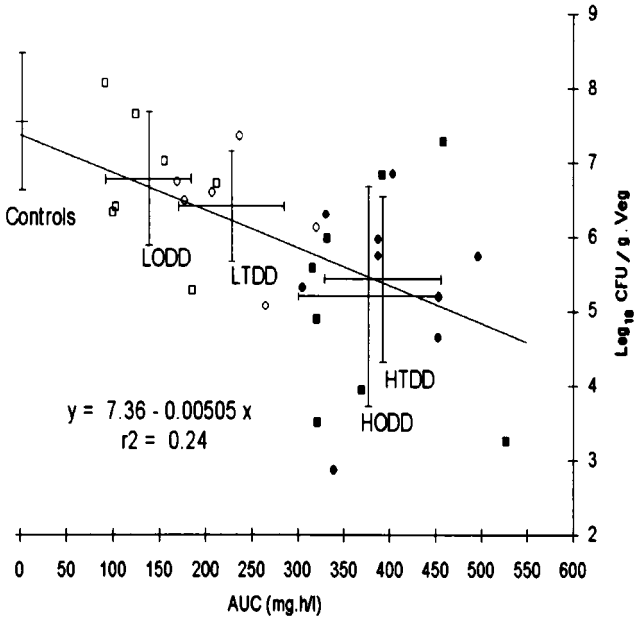

FIG. 3. Relation between AUC and surviving bacteria in vegetations after 24 h of amikacin treatment simulating human serum pharmacokinetics (elimination half-life, about 2 h). The control group (no treatment) is represented, but these animals were not included in the regression calculation. Each point represents an animal belonging to one of the four treated groups simulating the following doses: a single dose of 15 mg/kg (□), a single dose of 30 mg/kg (■), 15 mg/kg in three injections (○), and 30 mg/kg in three injections (●). For each treated group, a cross represents the mean and standard deviation of AUC (horizontally) and $\log_{10}$ CFU/g per vegetation (vertically).

same animals that are used to assess antibacterial effect. This would also provide better precision for analysis of the pharmacodynamic relationship through development of a correlation between AUC and effect.

We determined that the antibacterial effect of amikacin was linearly proportional to AUC and that the administration mode used (ODD or TDD) had no influence on the result. In fact, a more complete pharmacodynamic study of amikacin over a wide range of doses has shown a dose-effect relationship better described by a sigmoid curve (7, 18). However, the limited dose range studied here (simulation of 15 and 30 mg/kg/day) probably corresponds to the median, rectilinear portion of the general sigmoid curve, far below the asymptotic maximal effect. The doses we used are concordant with those reported by Craig et al. which were required to achieve 50% of the maximal effect in a group of animals with renal insufficiency in order to simulate human pharmacokinetics (7).

With respect to the therapeutic regimen, Potel et al. (28), using the same bacterial strain in the same model without simulation of human pharmacokinetics, found greater efficacy with fractionated dosage. Similar results have been reported by other authors for various infectious models and pathogens in animals with native pharmacokinetics (7, 17, 18, 26, 32, 35). However, once-daily dosage has proved at least as effective as fractionated dosage in models simulating 2-h elimination half-life in human serum either in vitro (2, 4, 13) or in vivo (7). A systematization of the divergences between these various studies should be based on the pharmacokinetic characteristics of the experimental models used. In other words, all these findings, as well as our findings versus those of Potel (28), seem to suggest the same rule for aminoglycosides: the slower the elimination, the more feasible it is to space out the doses to obtain the best efficacy. Finally, it may be asked whether determinants other than pharmacokinetic factors (notably bacteriologic ones) need to be considered in determining the intervals for the optimal aminoglycoside regimen. The present study based on a single bacterial strain could not explore this point.

Our results suggest that elimination half-life is a determinant factor in choosing the most efficient timing for aminoglycoside administration. Fractionated dosages or continuous infusion is more effective for studies with animals with a short elimination half-life, whereas ODD and TDD give equivalent results when normal human pharmacokinetics is simulated in the animal. In the latter case, AUC, as a linear predictive element for efficacy, was the same for both regimens in our study. This suggests that the maximal concentrations to be reached during ODD administration should be actually three times as high as those previously recommended for TDD (22). As recently reported in a wide clinical study involving an ODD regimen with gentamicin and tobramycin, such a peak level could be obtained without enhanced toxicity by using a 7-mg/kg/day dose instead of the conventional 4.5 mg/kg/day (24).

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