Identification of Factors Affecting In Vivo Aminoglycoside Activity in an Experimental Model of Gram-Negative Endocarditis

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Aminoglycoside bactericidal activity during the first 24 h of treatment probably is a determining parameter in the prognosis of severe gram-negative infections in immunocompromised patients. To identify the predictive factors involved in the definition of the best therapeutic regimen for Enterobacter cloacae and Serratia marcescens infections, we studied different gentamicin, tobramycin, and amikacin regimens by using an experimental model of rabbit endocarditis. Two factors appear to play an important role in predicting in vivo efficacy: (i) the level of in vivo bactericidal activity, which can differ widely from one aminoglycoside to another for the same bacterial strain and from one strain to another of the same species, and (ii) the critical serum drug concentration (CSC, in milligrams per liter), defined as the lowest serum antibiotic concentration capable of producing a significant CFU reduction ($P < 0.05$) in endocarditis vegetations 24 h after the beginning of a continuous infusion. Stepwise regression analysis showed that for gentamicin and $S$. marcescens, the area under the concentration-time curve above the CSC and then the time above the CSC are the determining parameters for efficacy ($R = 0.69; F = 13.5; P = 0.001$), whereas for amikacin and $S$. marcescens, the time above the CSC and then the area under the concentration-time curve above the CSC predict efficacy ($R = 0.74; F = 24.0; P = 0.0001$). The lowest CSC is that of amikacin (about 8 mg/liter); those of gentamicin and tobramycin are about 15 mg/liter. In severe $S$. marcescens infections, intermittent amikacin dosing offers excellent bactericidal activity within the first 24 h.

It has been seen in numerous clinical and experimental studies that once-daily dosing of an aminoglycoside is at least as effective as administration of the same quantity of drug as divided doses (1, 11, 13). However, most of these studies examined only a limited number of bacterial species ($Escherichia$ $coli$, $Klebsiella$ $pneumoniae$, and $Pseudomonas$ $aeruginosa$), and all compared the effects of several treatment regimens (including once-daily dosing) with the same aminoglycoside (4, 13). Among gram-negative bacteria, $Enterobacter$ and $Serratia$ species have assumed increasing importance in the last few years (17, 26). In one French series, $Enterobacter$ and $Serratia$ species accounted for 21 of 134 (15.6%) cases of gram-negative septicemia recorded in 1989 (2).

Investigation of the in vivo bactericidal activities of antibiotics in the first 24 h after administration is probably an important measure, with potentially crucial implications for human use, principally in neutropenic (or aplastic) patients.

In a recent experimental study (22), we showed that the same doses of gentamicin, tobramycin, and amikacin had different effects in a model of $Serratia$ $marcescens$ endocarditis, despite similar pharmacokinetics. In brief, the same single dose administered as an intravenous (i.v.) bolus injection exhibited the best efficacy for gentamicin, whereas the same dose administered as a continuous i.v. infusion exhibited the best efficacy for amikacin. Furthermore, one in vitro study (12) has suggested that higher initial peak concentrations of aminoglycosides are necessary to achieve bactericidal activity for $S$. $marcescens$ and $Enterobacter$ $cloacae$, compared with most susceptible species, such as $E$. $coli$. Thus, the recommended dosage regimens for aminoglycosides are not necessarily identical from one species to another.

Finally, although many authors (3, 4, 10, 18, 25) have demonstrated a good correlation between some pharmacokinetic parameters and in vivo bactericidal activities of aminoglycosides, these studies usually used an in vitro parameter (usually the MIC) to establish the correlation.

In an attempt to identify, at least in part, the factors affecting in vivo aminoglycoside activity, we chose to study the in vivo effects of gentamicin, tobramycin, and amikacin at various dosage regimens on both $E$. $cloacae$ and $S$. $marcescens$ experimental endocarditis. We tried to confirm the importance of the in vitro killing rate in the efficacy of a single i.v. bolus dose, as previously shown in the same model of $E$. $coli$ endocarditis (23). The second parameter which was investigated was the critical serum drug concentration (CSC, in milligrams per liter), defined as the lowest serum antibiotic concentration able to achieve significant in vivo bactericidal activity 24 h after the beginning of a continuous i.v. infusion in the $S$. $marcescens$ endocarditis model. Thus, we expected to explain some of the differences in the results reported in the literature regarding the optimum doses of aminoglycosides (11, 15) by studying the early bactericidal activities of three aminoglycosides in experimental gram-negative endocarditis, representing a model of severe intravascular infection without host defenses at the site of infection.

(This work was presented in part at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, Calif., 23 to 26 October 1988 [21a].)
MATERIALS AND METHODS

Organisms. One strain of S. marcescens (HN229) and two strains of E. cloacae (E474 and E475) were cultured from the urine (HN229), blood (E474), or lungs (E475) of hospitalized patients. All strains were resistant to rabbit serum.

Antibiotics. The three aminoglycosides studied were tobramycin (Eli-Lilly), gentamicin (Scherer-Plough), and amikacin (Bristol-Myers-Squibb).

In vitro studies. (i) Antibiotic susceptibility tests. The MIC of each antibiotic was determined with Mueller-Hinton broth supplemented with Ca\(^{2+}\) (50 µg/ml) and Mg\(^{2+}\) (25 µg/ml) in 200-µl wells and with an inoculum of 10\(^5\) CFU/ml in the exponential growth phase. The MIC was defined as the lowest concentration of antibiotic capable of inhibiting all visible growth after 18 h of incubation. After 24 h, a subculture was transferred to Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.). The MBC corresponded to the lowest concentration of drug permitting 0.1% of the bacteria to survive and was determined by placing 1-µl portions of cultures, by use of a Steers replicator, on agar plates with 3% polyglycol sulfonic acid sodium salt (SPS; Sigma) (19).

(ii) Killing curves. Time-kill curves were drawn for each antibiotic at 12 concentrations in Mueller-Hinton broth: 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 µl/g of infection. For each concentration, the antibiotics were incubated in microtubes (1-ml tubes; Macrowell; Skatron, Lier, Norway) with an inoculum of 10\(^5\) S. marcescens or E. cloacae stationary-phase cells per ml. Surviving bacteria were counted in each tube after 1.5, 3, 5, and 24 h of incubation by a semiautomatic dilution micromethod involving an automatic 96-well dispenser (Skatron) and a Steers replicator distributing 2 ± 0.5 µl of each dilution onto an agar plate supplemented with 3% SPS, avoiding a carryover phenomenon. After 24 h of incubation, the first dilution with 5 to 30 colonies was read, and the colony count was multiplied by the dilution factor. The standard error of this count was 0.2 log\(_{10}\) CFU/ml. The sensitivity limit of detection was 2.4 log\(_{10}\) CFU/ml (7). Expression of the results was described in a previous work (9). In brief, the number of surviving bacteria was expressed as log\(_{10}\) CFU per milliliter after each incubation period (1.5, 3, 5, and 24 h). Only the early phase of the killing curve (i.e., the first 5 h) was plotted on a graph. For each concentration of antibiotic, the area under the curve for the surviving bacteria between 0 and 5 h (AUC of surviving bacteria) was calculated, and a percentage of the hypothetical area for the reference inoculum if no bactericidal activity had occurred was then determined. This parameter was called the index of surviving bacteria (ISB) and was calculated as follows: ISB (percent) = (AUC of surviving bacteria/AUC of inoculum) x 100. This calculation allowed us to represent the 5-h killing curves for all concentrations studied on a two-dimensional graph.

Experimental endocarditis. In vivo studies were carried out on New Zealand White female rabbits (age, 12 to 15 weeks; weight, 2.5 to 3.5 kg). The animals were kept in individual cages and allowed free access to food and water throughout the experiment. Left ventricular endocarditis was induced as described previously (21, 22). At 24 h after the introduction of a polyethylene catheter through the aortic valve, each rabbit received 1 ml of a suspension containing 10\(^7\) organisms per ml, injected through the marginal ear vein.

(i) Experimental design. (a) In vivo efficacy of a single i.v. bolus dose. In a previous work (23), we showed, by using E. coli experimental endocarditis, that the in vitro killing rate was predictive of the in vivo efficacy of a single i.v. dose, i.e., the higher the in vitro killing rate, the lower the single i.v. dose required to achieve a significant reduction in the CFU in the vegetations 24 h after the single i.v. bolus dose. This dose was the minimal effective dose (MED, in milligrams per kilogram). Since the two strains of E. cloacae (E474 and E475) differed in terms of in vitro killing rates following tobramycin and gentamicin, a single 48-mg/kg dose of each of these antibiotics was administered to two groups of animals with E. cloacae E474 or E475 endocarditis to test how well the observed differences in in vitro bacterial killing predicted in vivo activity.

In the S. marcescens endocarditis model, various doses of gentamicin, tobramycin, and amikacin were tested as bolus injections to achieve the MED. The doses of amikacin and tobramycin tested were 48 and 72 mg/kg. Since, in the case of gentamicin, a dose of 48 mg/kg had produced a maximum in vivo antibacterial effect at 24 h (22), animals treated with 24 or 48 mg/kg were sacrificed 6 h after the i.v. bolus dose to ascertain how early bacterial killing occurred in vivo.

(b) Determination of the CSC. To explain the differences previously observed in the model of S. marcescens endocarditis during continuous 24-h infusions of identical doses of tobramycin, amikacin, and gentamicin (22), we determined the in vivo CSC of each antibiotic by using the same model. The doses were chosen in light of previous results showing that a dose of 48 mg/kg was ineffective for tobramycin and gentamicin, while a maximum effect was observed with amikacin. This dose corresponded to a steady-state concentration of about 10 µg/liter. At 48 h after inoculation, the animals were randomized to one of the following groups: each antibiotic being administered as a continuous infusion over 24 h: amikacin at 24 mg/kg or gentamicin or tobramycin at 72 mg/kg.

(c) In vivo effect of multiple doses of amikacin on S. marcescens endocarditis. Since the continuous infusion of amikacin over 24 h appeared more effective in the S. marcescens endocarditis model than the same dose administered as a single bolus injection (22), various split-dose regimens (6 mg/kg every 6 h, 12 mg/kg every 6 h, 12 mg/kg every 12 h, and 24 mg/kg every 12 h) were tested in some animals to verify that a 24-h continuous i.v. infusion was predictive of the efficacy of a split-dose regimen.

(ii) Evaluation of treatment. The effect of each treatment was evaluated 24 h after the therapeutic regimen assigned was begun. The animals were sacrificed with an i.v. bolus injection of thiopental. The heart was removed, and vegetations were excised and rapidly rinsed in sterile saline. Some of the vegetations were weighed and homogenized in a Thomas Teflon pestle tissue homogenizer with 0.5 ml of sterile saline. Serial dilutions of 0.05 ml of the homogenate were spread by use of a Spiral System (Interscience) and quantitatively cultured for 24 h at 37°C on Trypticase soy agar plates containing 3% SPS, avoiding a carryover phenomenon. Bacterial titers were expressed as log\(_{10}\) CFU per gram of vegetation. We were able to detect quantities as small as 20 CFU/ml of homogenate, and the value integrated for the calculation of the mean bacterial titer took into account the weights of vegetations. Part of each vegetation was frozen prior to the antibiotic assays.

(iii) Pharmacokinetic studies. Blood samples were taken from three animals in each group to determine the plasma concentration-time curve for each therapeutic regimen. Animals from the following different bolus groups were studied: 24 mg/kg for gentamicin; 6, 12, 24, and 72 mg/kg for...
amikacin; and 72 mg/kg for tobramycin. For the continuous infusion groups, blood samples were taken after 6 h (steady state) from animals receiving 24 mg of amikacin per kg or 72 mg of tobramycin or gentamicin per kg.

The blood samples were obtained by placement under local anesthesia of a catheter in the left femoral artery in rabbits receiving a bolus injection and by acute femoral puncture in animals receiving a continuous infusion. The samples were immediately centrifuged, and the serum was frozen until the time of the assays.

The various pharmacokinetic parameters (half-life, total AUC, time or AUC above the CSC, and MIC) were calculated by use of a monocompartmental model with the aid of our own computer program.

(iv) Antibiotic assays. Serum antibiotic concentrations for each antibiotic regimen were determined by use of a microbiological assay with Bacillus subtilis ATCC 6633. The range of measurable concentrations with this strain was 0.06 to 1 μg/ml for all three antibiotics.

After being weighed and homogenized with 0.3 ml of 0.1 M phosphate buffer, the vegetations were centrifuged and the supernantant fluid was sampled for a microbiological assay. The same strain of B. subtilis as that listed above was used.

(v) Statistical evaluation. An analysis of variance (Super ANOVA; Abacus Concepts, Inc.) and Scheffe’s S test were performed to compare the bacterial titers measured in treated animals versus controls. A normality test (Statworks; Cricket Software, Inc.) was performed first to establish the feasibility of the statistical procedure.

The number of sterile vegetations in each experimental group was compared with that in the control group by a χ² test with Yates’ continuity correction. Linear and then stepwise regression analyses were performed to study the relationship between the various pharmacokinetic parameters and in vivo antibacterial effects, expressed as Δlog₁₀ CFU per gram of vegetation in experimental groups versus controls (Statview; Abacus Concepts, Inc.). The analysis focused first on the mean results for each group (mean Δlog₁₀ CFU per gram of vegetation in experimental groups versus controls). Gentamicin and amikacin were subjected to a separate analysis by examining the relationship between the individual results (Δlog₁₀ CFU per gram of vegetation) and the pharmacokinetic parameters.

RESULTS

In vitro studies. (i) Antibiotic susceptibility tests. The MICs (and MBCs) for the S. marcescens strain studied were 0.5 μg of gentamicin per ml and 1 μg of tobramycin or amikacin per ml. The MICs (and MBCs) for both of the E. cloacae strains studied were 0.5 μg of gentamicin or tobramycin per ml and 1 μg of amikacin per ml.

(ii) Killing curves. The 5-h bacterial killing curves obtained after exposure of the S. marcescens strain to the three aminoglycosides are shown in Fig. 1 for each concentration studied. Each concentration at which the ISB was greater than 100% corresponds to regrowth. At all concentrations above 1 μg/ml, the percentage of surviving bacteria at 5 h (expressed as the ISB) was consistently lower for gentamicin than for amikacin or tobramycin.

Figure 2 shows the differences observed with tobramycin and gentamicin for the two strains of E. cloacae, strain E475 being killed more rapidly than strain E474 at concentrations between 2 and 32 μg/ml. These results represent the mean of three consecutive experiments. The differences observed were far above the standard deviation of the method of counting (see Materials and Methods) and appeared highly significant.

In each experiment, it was possible to confirm that the MICs for the surviving bacteria were the same as those for the parental strain with respect to the tested antibiotics.

In vivo studies. (i) In vivo efficacy of a single i.v. bolus dose. For E. cloacae endocarditis, the same bolus dose of gentamicin or tobramycin (48 mg/kg) produced a significant reduction in the CFU of strain E475 but not strain E474 (Table 1).

For S. marcescens endocarditis, we showed in an earlier study (22) that the same bolus dose of an aminoglycoside (48 mg/kg) had different effects, depending on the compound used, gentamicin producing the maximum effect (2.7 ± 0.2
log$_{10}$ CFU/g of vegetation; 6 of 9 sterile vegetations) and tobramycin producing practically no effect (6.8 ± 1.9 log$_{10}$ CFU/g of vegetation; 0 of 10 sterile vegetations), like amikacin (7.5 ± 1.3 log$_{10}$ CFU/g of vegetation; 0 of 10 sterile vegetations). In the present study, we were able to demonstrate that an antibiotic effect could be achieved in vivo with a higher dose (72 mg/kg) of tobramycin or amikacin: 4.3 ± 1.7 log$_{10}$ CFU/g of vegetation ($P = 0.056$ versus controls) for tobramycin and 4.4 ± 2.1 log$_{10}$ CFU/g of vegetation ($P = 0.03$ versus controls) for amikacin; this result revealed a dose-related killing effect of these two drugs on the S. marcescens strain.

Since the effect of 48 mg of gentamicin per kg was virtually maximal after 24 h, a number of animals were sacrificed 6 h after the same dose was administered as a bolus injection to evaluate in vivo bacterial killing by this antibiotic. Bactericidal activity was already very marked 6 h after a dose of 48 mg/kg (3.5 ± 1.9 log$_{10}$ CFU/g of vegetation; $P = 0.003$), whereas it was not significant after a dose of 24 mg/kg (5.1 ± 2.5 log$_{10}$ CFU/g of vegetation).

(ii) **Determination of the CSC for the S. marcescens strain.** The results obtained for determination of the CSC for the S. marcescens strain at the various concentrations studied are shown in Table 2. The lowest CSC was that of amikacin, at about 8 mg/liter. The lowest concentration of this antibiotic studied (5.3 mg/liter) produced a nonsignificant ($P = 0.11$ versus controls) antibacterial effect (4.8 ± 2.2 log$_{10}$ CFU/g of vegetation).

### Table 1. In vivo effect of the same dose of tobramycin or gentamicin (48 mg/kg) 24 h after i.v. bolus administration on E. cloacae E474 or E475 endocarditis

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Strain</th>
<th>Mean ± SD vegetation titer (log$_{10}$ CFU/g of vegetation)</th>
<th>No. of sterile vegetations/total no. of vegetation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>E474</td>
<td>8.5 ± 0.7</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>E475</td>
<td>8.9 ± 0.5</td>
<td>0/8</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>E474</td>
<td>7.8 ± 0.8</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>E475</td>
<td>6.1 ± 1.4</td>
<td>1/9</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>E474</td>
<td>7.3 ± 1.6</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>E475</td>
<td>5.6 ± 2.0</td>
<td>0/9</td>
</tr>
</tbody>
</table>

$^a$ $P = 0.008$ versus control E475 (Sheffe’s S test).
$^b$ $P = 0.001$ versus control E475 (Sheffe’s S test).

### Table 2. In vivo effect of various steady-state concentrations of gentamicin, tobramycin, or amikacin maintained in serum for 24 h by a continuous infusion of 24, 48, or 72 mg/kg on S. marcescens endocarditis

<table>
<thead>
<tr>
<th>Treatment group (mg/kg)</th>
<th>Steady-state conen in serum (mg/liter, mean ± SD)</th>
<th>Mean ± SD vegetation titer (log$_{10}$ CFU/g of vegetation)</th>
<th>No. of sterile vegetations/total no. of rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>0/11</td>
</tr>
<tr>
<td>Gentamicin (48)</td>
<td>7.9 ± 2.1</td>
<td>7.8 ± 0.4</td>
<td>0/6</td>
</tr>
<tr>
<td>Gentamicin (72)</td>
<td>15.0 ± 8.0$^b$</td>
<td>6.4 ± 1.4</td>
<td>0/6</td>
</tr>
<tr>
<td>Tobramycin (48)</td>
<td>9.2 ± 1.9</td>
<td>2.5 ± 0.2$^b$</td>
<td>5/6$^c$</td>
</tr>
<tr>
<td>Tobramycin (72)</td>
<td>16.8 ± 3.0$^a$</td>
<td>2.9 ± 0.9$^b$</td>
<td>4/6$^c$</td>
</tr>
<tr>
<td>Amikacin (24)</td>
<td>5.3 ± 1.5</td>
<td>4.8 ± 2.2</td>
<td>3/9</td>
</tr>
<tr>
<td>Amikacin (48)</td>
<td>8.0 ± 1.9$^a$</td>
<td>3.6 ± 2.0$^b$</td>
<td>5/8$^c$</td>
</tr>
</tbody>
</table>

$^a$ CSC (see the text).
$^b$ $P < 0.01$ versus control (Sheffe’s S test).
$^c$ $P < 0.05$ versus control (χ$^2$ test with Yates’ correction).

### Table 3. In vivo effect of various doses of amikacin administered for 24 h in the experimental model of S. marcescens endocarditis

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Regimen$^a$</th>
<th>Cumulative dose (mg/kg/24 h)</th>
<th>Mean ± SD vegetation titer (log$_{10}$ CFU/g of vegetation)</th>
<th>No. of sterile vegetations/total no. of rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td></td>
<td></td>
<td></td>
<td>0/11</td>
</tr>
<tr>
<td>6</td>
<td>q6h</td>
<td>24</td>
<td>4.2 ± 2.7$^b$</td>
<td>6/12$^c$</td>
</tr>
<tr>
<td>12</td>
<td>q6h</td>
<td>48</td>
<td>3.5 ± 1.4$^b$</td>
<td>5/9$^c$</td>
</tr>
<tr>
<td>12</td>
<td>q12h</td>
<td>24</td>
<td>7.8 ± 0.9</td>
<td>0/6</td>
</tr>
<tr>
<td>24</td>
<td>q12h</td>
<td>48</td>
<td>3.0 ± 0.5$^b$</td>
<td>2/7</td>
</tr>
<tr>
<td>48</td>
<td>q24h</td>
<td>48</td>
<td>7.5 ± 1.3</td>
<td>0/10</td>
</tr>
<tr>
<td>72</td>
<td>q24h</td>
<td>72</td>
<td>4.4 ± 2.1$^b$</td>
<td>4/9$^d$</td>
</tr>
</tbody>
</table>

$^a$ q, every.
$^b$ $P < 0.01$ versus control (Sheffe’s S test).
$^c$ $P < 0.05$ versus control (χ$^2$ test with Yates’ correction).
$^d$ $P = 0.05$ versus control (χ$^2$ test with Yates’ correction).

### Predictive value of pharmacokinetic parameters. Simple linear regression analysis of the mean Δlog$_{10}$ CFU per gram of vegetation in each of the test groups against the various pharmacokinetic parameters studied (AUC, AUC above the CSC, AUC above the MIC or MBC, time above the CSC, and time above the MIC or MBC) revealed that only the parameter time above the CSC was significantly correlated with in vivo bactericidal activity ($R = 0.55; P = 0.027$). An analysis of the individual values for Δlog$_{10}$ CFU per gram of vegetation separately for gentamicin and amikacin proved to be much more discriminating. In the case of gentamicin, simple regression analysis revealed that the parameter with the best correlation was AUC above the CSC ($R = 0.61; P = 0.0002$); this was followed by AUC above the MIC ($R = 0.45; P = 0.008$), time above the CSC ($R = 0.39; P = 0.027$), and total AUC ($R = 0.38; P = 0.032$). Time above the MIC was not significantly correlated ($R = 0.20; P = 0.25$). For amikacin, the best-correlated parameters were time above the CSC ($R = 0.41; P = 0.006$) and time above the MIC ($R = 0.64; P = 0.0001$); these were followed by AUC ($R = 0.30$);
TABLE 4. Pharmacokinetic parameters corresponding to each i.v. bolus dose

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dose (mg/kg)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Half-life (min)</td>
<td>AUC* (mg · h · liter⁻¹)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>6  50 ± 5 28 ± 3</td>
<td>8.6 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>12  43 ± 3 49 ± 3</td>
<td>26.4 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>24  50 ± 3 115 ± 7</td>
<td>82.0 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>48  72 ± 4 332 ± 18</td>
<td>274.0 ± 15.0</td>
</tr>
<tr>
<td></td>
<td>72  77 ± 5 533 ± 34</td>
<td>465.0 ± 30.0</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>48  67 ± 9 301 ± 4</td>
<td>217.3 ± 31.8</td>
</tr>
<tr>
<td></td>
<td>72  70 ± 4 484 ± 27</td>
<td>384.0 ± 22.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>24  63 ± 3 146 ± 7</td>
<td>80.0 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>48  65 ± 5 300 ± 23</td>
<td>216.3 ± 16.5</td>
</tr>
</tbody>
</table>

* Calculated from time zero to the last measurable point and then extrapolated to infinity. For animals receiving an injection every 6 h (or every 12 h), the AUC was calculated from 0 to 6 h (or 12 h) and then multiplied by 4 (or 2) to obtain the AUC over 24 h.

For amikacin, the calculated AUC was 79 ± 8 mg · h · liter⁻¹, and the time above the median effective concentration (MED) was 79 ± 8 min.

For tobramycin, the calculated AUC was 111 ± 8 mg · h · liter⁻¹, and the time above the MED was 111 ± 8 min.

For gentamicin, the calculated AUC was 169 ± 8 mg · h · liter⁻¹, and the time above the MED was 169 ± 8 min.

P = 0.03). Stepwise regression analysis revealed that the best correlation was shown by AUC above the CSC and then time above the CSC for gentamicin (R = 0.69; F = 13.54; P = 0.001), compared with time above the CSC and then AUC above the CSC for amikacin (R = 0.74; F = 24.07; P = 0.0001).

**DISCUSSION**

The mode of administration of aminoglycosides has been a subject of debate for many years. A number of clinical studies in the late 1970s (8, 14) encouraged the use of continuous infusion in combination with a beta-lactam antibiotic for neutropenic patients. However, these studies were not comparative. Nevertheless, in the study of Keating et al. (14), the combination of carbenicillin and amikacin as a continuous infusion proved more effective than that of carbenicillin and gentamicin as a continuous infusion for the treatment of septicemia in neutropenic patients, although it was not possible to establish the respective roles of the aminoglycoside concentrations or that gram-negative bacilli are less susceptible to gentamicin (in terms of the MIC). Our results seem to show that the in vivo activities of aminoglycosides (and the best dosage schedules) depend on many factors, including the bacterial species, the strain studied, the aminoglycoside used, and probably the site of infection. To identify the factors predictive of the best dosage regimen for a severe gram-negative intravascular infection, we determined in vitro (killing rate) and in vivo (CSC) parameters to explain the differences in the in vivo activities of three aminoglycosides.

In the *E. cloacae* endocarditis model, despite the similar MICs of the two compounds used (gentamicin and tobramycin), the slight difference in the in vivo activity of a single i.v. bolus injection (48 mg/kg) on the two strains (E474 and E475) could have been related to the higher killing rate in vitro (expressed as the ISB) of these two drugs for strain E475 than for strain E474. This result is in agreement with that of a previous study (23), in which a good correlation was demonstrated between the in vivo killing rate and the in vivo effect of a single dose of antibiotic in an *E. coli* endocarditis model. Similarly, the better in vivo activity of gentamicin than of tobramycin or amikacin on the strain of *S. marces-

cens* was probably responsible for the low MED of the former compound (48 mg/kg for gentamicin versus 72 mg/kg for amikacin and tobramycin). Nevertheless, determination of the CSC seemed to be necessary to establish a valuable in vivo dose-effect relationship, allowing recommendation of the best dosage regimen for an aminoglycoside in a given bacteriological and clinical situation. In fact, as has been reported in several studies of thigh infection in neutropenic mice by Craig and colleagues (3, 16, 25), time above the MIC is most often the best predictor of the in vivo efficacies of aminoglycosides for the majority of tested strains, as long as the dosage interval is over 6 h. Under these conditions, in which the administered substance is rapidly eliminated by small animals, high, extremely bactericidal aminoglycoside concentrations cannot be maintained for a sufficient time to produce an in vivo bactericidal effect. In our study, AUC above the CSC was still important for the three antibiotics tested but was more important for gentamicin than for amikacin. The fact that gentamicin had the lowest MED (48 mg/kg) for *S. marcescens* is explained by the much higher bacterial killing rate with this drug than with amikacin or tobramycin. Sacrifice of the animals treated with gentamicin after 6 h confirmed that most of the bactericidal activity took place in the first 6 h, during which concentrations in excess of the CSC were observed in the serum for about 3 h. In the case of amikacin, the same dose of antibiotic resulted in no bactericidal activity at the end of 24 h since, although the concentrations in serum exceeded the CSC for 6 h, this period was inadequate to allow the expression of a bactericidal effect slower than that of gentamicin. The results for the multiple doses of amikacin were in agreement with those obtained by Craig et al. for mice with *S. marcescens* thigh infections (4). The cumulative 24-h dose required to achieve a bactericidal effect in vivo increased with the length of the dosage intervals (24, 48, and 72 mg/kg for intervals of 6, 12, and 24 h, respectively). It should be noted that a possible postantibiotic effect was not investigated in our model, since the residual concentrations in the vegetations were always equal or superior to the MIC, thus excluding the possibility of regrowth as well.

Studies comparing the relative efficacies of several aminoglycosides in the same model of gram-negative infection are rare, while experimental or clinical studies comparing the efficacy of a once-daily injection with that of intermittent dosing yield divergent results (1, 13, 20, 24, 25). One of the reasons for these differences could be a failure to take into account the in vitro bacterial killing rate, which seemed to play a major predictive role in the present study for both *E. cloaca* and *S. marcescens*. For three aminoglycosides with comparable pharmacokinetics, it was found that the higher the killing rate, the lower the MED. Finally, in the absence of a comparative study, we would have shown that, in a severe *S. marcescens* infection, amikacin was more effective as a continuous infusion than as a bolus injection, that gentamicin was more effective as a bolus injection than as a continuous infusion, and that tobramycin was equally effective in both modes of administration. By taking into account CSC and in vitro bacterial killing rate, the observed differences could be explained; a valuable dose-effect relationship was based on different predictive factors for the in vivo efficacies of gentamicin and amikacin in this model (AUC above the CSC for gentamicin and time above the CSC for amikacin). Measurement of these two parameters (in vitro killing rate expressed as ISB and CSC) should, in our opinion, be included in experimental studies of the pharmacodynamics of antibiotics. Nevertheless, we are not sure
whether a CSC defined for one infected site is predictive for another one. Investigation of bactericidal activity during a period as short as 24 h offers several advantages. (i) The in vivo concentration-effect relationship can be studied with greater precision than during longer observation periods (24), since possible initial differences between two antibiotic regimens may vanish during a treatment lasting several days. (ii) It is probable that the activities of antibiotics (especially aminoglycosides) in the first hours of a severe gram-negative infection in an immunocompromised patient constitute an important prognostic factor, justifying the search for the best, well-adapted dosing regimen.

The limitations of animal models in the comparative study of once-daily versus intermittent dosing regimens have recently been underlined by Craig and coworkers (4) and involve the rapid clearance of antibiotics in small animals, resulting in a half-life shorter than that in humans. This parameter probably plays an important role in the relationship between the 24-h cumulative dose and efficacy, and the present study confirms the high predictive value of time above the CSC for the level of in vivo bactericidal activity. In our study, the most rapid bactericidal effect of gentamicin administered as a single i.v. bolus injection was achieved with doses producing concentrations not recommended for human treatment. It is thus by no means certain that a favorable conclusion can be drawn from this study with regard to once-daily dosing of gentamicin. Similarly, it is possible that with a longer half-life, once-daily amikacin can achieve efficacy in the model of S. marcescens endocarditis equivalent to that observed with a continuous infusion or intermittent administration. Nevertheless, it must be stressed that the CSC of amikacin (5.3 to 8 μg/ml) is consistent with the concentrations achieved in humans under normal dosage schedules. In fact, a continuous infusion of 15 mg of amikacin per kg in humans produces steady-state serum drug concentrations of about 10 μg/ml, comparable to the concentrations that we observed in rabbits with 48 mg/kg (6). Thus, a possible conclusion that can be extrapolated to humans with severe S. marcescens infections is that amikacin, administered as three or four daily doses or as a continuous infusion, displays outstanding efficacy within 24 h of administration. Shorter intervals are probably not suitable, because of the possibility of a down-regulation phenomenon, responsible for adaptive resistance in the 6 h following a brief exposure to an aminoglycoside (5).

In conclusion, two factors affect the in vivo efficacies of the aminoglycosides and probably explain, at least in part, the discrepancies reported in the literature for the optimal dosing regimens of these drugs: the in vitro killing rate, which may be measured at several concentrations, and the in vivo CSC, which can be much higher than the MIC. Further studies are needed to examine whether there is a relationship between these early bactericidal effects and the ultimate treatment outcome of severe gram-negative sepsis.

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