Efficacy of Intermittent Versus Continuous Administration of Netilmicin in a Two-Compartment In Vitro Model

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Several aminoglycoside dosage regimens were studied in a kinetic in vitro model. Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus were exposed in serially placed artificial capillary units to netilmicin concentrations that changed based on human two-compartment pharmacokinetics. The same total dose per 24 h was administered as a continuous infusion (3.7 µg/ml) or in 1-h infusions given every 24 (24 µg/ml) or 8 h (8 µg/ml). The once daily administration showed the best response in terms of either faster killing of E. coli, K. pneumoniae, and S. aureus or greater reduction of the inocula of P. aeruginosa. After 28 h of treatment, however, all regimens reduced the nonpseudomonads by more than 99.99%, whereas all three P. aeruginosa strains regrew to >10^8 CFU/ml due to selection of resistant subpopulations. In contrast to the bactericidal effect of the first dose, no killing occurred after subsequent doses if the ratio of peak drug concentration to MIC was low (≤6). These results support the concept of administering high doses of aminoglycosides once every 24 h.

Most standardized in vitro methods for determining antibiotic activity involve the use of static concentrations of these drugs in contact with a given bacterial inoculum for 18 to 20 h (11, 27). However, during the treatment of a clinical or in vivo experimental infection, the bacteria at the site of infection are exposed to continuously changing drug concentrations. The past 5 years have seen the development of several in vitro models for testing antibiotic activity that consider pharmacokinetic principles (15, 16, 19, 23, 25, 26; B. Ledergerber, A. Hugéntobler, J. Blaser, R. Lüthy, and M. Anliker, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 898, 1983). The one- and two-compartment models described previously allow the exposure of a single bacterial culture to changing drug concentrations. The two-compartment capillary model presented here is designed for multiple cultures and did not result in dilution of the bacterial inoculum. This model represents several modifications of the artificial capillary unit model described previously (29) and allowed for continuous drug elimination, homogeneous drug concentration in the peripheral compartments, and the study of several organisms simultaneously.

The optimal administration of antibiotics has been the subject of much debate (3, 7, 9, 13, 20; T. A. Drake, H. F. Chambers, and M. A. Sande, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 835, 1982). Some authors of clinical studies have advocated continuous administration of aminoglycosides (3, 9), but other reports have suggested that intermittent administrations are more effective than continuous infusions (13; 22nd ICAAC, abstr. no. 835). In a recent study, Powell et al. compared once daily aminoglycoside dosing with continuous infusion in animal models and also in 52 patients with cystic fibrosis (20). They suggested that intermittent dosing, resulting in infrequent, large, maximum serum concentrations (40 µg of tobramycin per ml) may be less toxic and as effective as more frequent dosing.

In the present paper, the two-compartment capillary model was used to study the effects of identical total daily doses of netilmicin, administered as continuous or intermittent infusions in several dosage schedules, against strains of Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, and Klebsiella pneumoniae.

MATERIALS AND METHODS

Description of the model. A two-compartment capillary model was designed to expose bacteria in the peripheral compartments to changing concentrations of antibiotics without dilution of the inoculum (Fig. 1). The basic unit of the peripheral compartment was the Vitafiber artificial capillary unit (model 3P-10; Amicon Corp., Lexington, Mass.). For each experiment, up to six units were placed in series. Each capillary unit consisted of a polycarbonate chamber through which ran a bundle of 150 artificial capillaries. These capillaries were hollow polysulfone fibers with an internal diameter of 0.2 mm. Their porous walls were permeable to molecules smaller than 10,000 daltons, with a total membrane area of ca. 60 mm². Thus, most antibiotics penetrated this interface from the central to the peripheral compartments and back again, but the passage of large proteins, bacteria, or blood cells was prevented.

Bacteria were placed in the chambers external to the capillary bundles. The volume of this space was extended by a closed loop of tubing to a total of 10 ml. A peristaltic pump (Tygon, 5-50-HL; Norton Corp., Akron, Ohio) circulated the contents of the peripheral compartment (bacteria, antibiotic, medium) at a constant rate of 3 ml/min to ensure homogeneous concentrations of bacteria and antibiotic. Two rubber septum fittings were placed in this circuit to allow for inoculation and sterile sampling by syringe aspiration (0.3 ml per sample).

The central compartment remained sterile and had a total volume of 76 ml. It included the internal volume of the capillaries plus the central reservoir and the connecting silicone rubber tubing (Silastic; Dow Corning Corp., Midland, Mich.). The central reservoir was fitted with a mag-
FIG. 1. Schematic of the two-compartment kinetic model for multiple cultures. Several isolated plastic chambers containing bacteria were placed in series and perfused with antibiotic through selectively permeable artificial capillary bundles. A drug was administered to the central compartment and then exponentially removed to continuous dilution with drug-free medium.

The concentration of antibiotics in the central compartment decreased according to first-order kinetics by continuous dilution and elimination. Sterile, drug-free broth was pumped into the reservoir at a flow rate which was set based on the half-life of the drug under study. To keep the volume of the system constant, broth which contained antibiotic was eliminated from the reservoir at the same rate. The antibiotic dissolved in broth was pumped from the central reservoir to the capillary units and diffused through the porous walls of the fibers to and from the peripheral compartments.

In practice, the entire system was autoclaved before use and filled with sterile Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.), supplemented with magnesium (25 μg of Mg2+/ml) and calcium (50 μg of Ca2+/ml) to approximate the free cation concentration in human serum (6). During experiments, the entire system including the pumps was housed within an incubator at 37°C, except for the infusion pump, which was kept outside at room temperature. The artificial capillary units were reusable after cleaning with trypsin–70% ethanol–water.

Drug kinetics. The kinetics of this model mimicked the distribution and first-order elimination of aminoglycoside pharmacokinetics based on in vivo data (1). The elimination half-life of netilmicin (t1/2) in this system was set at 2.2 h, based on the serum kinetics in young adults with normal renal function (1). This was achieved by setting the elimination pump of the system at a clearance rate (Cl) which was defined as follows:

\[
Cl = \ln 2 \frac{V_{tot}}{t_{1/2}}
\]

where \(V_{tot}\) is the total volume of distribution, including that of the central compartment plus all peripheral compartments (Fig. 1) and \(\ln 2\) is the natural logarithm of 2. The doses of netilmicin were determined by the desired nominal peak concentration (1, 8, or 24 μg/ml) achieved at the end of the first 60 min of infusion. The second and subsequent doses were identical to the first one. The nominal peak concentration was defined as the maximum concentration which would be found in a one-compartment model of a total volume equal to the total volume of the two-compartment model used in our present experiments. Because of the two-compartment kinetics of this model, the real peak concentration was higher than this theoretical nominal value in the central compartment and lower than the nominal value in the peripheral compartments (Fig. 2).

The amount of drug administered per 60 min of infusion that was necessary to obtain the desired nominal peak concentration was greater than would be calculated for a bolus injection into the total volume of distribution. Equation 2 takes into account the ongoing clearance during the time of infusion (\(\Delta t\)) to calculate the dose (\(D\)) needed to achieve the nominal maximum concentration (\(C_{max}\)) at the end of the first infusion:

\[
D = C_{max} Cl \Delta t (1 - e^{-Cl \Delta t/V_{tot}}) = C_{max} Cl \Delta t (1 - e^{-Cl \Delta t/V_{tot}})
\]

The dose of netilmicin administered for a total volume of distribution of 136 ml was 3.8 mg/24 h (27.9 μg/ml), except for the low-dose (every-8-h \([q8h]\)) regimen, for which only one-eighth of this dose was given (3.5 μg/ml).

Dosage regimens. In all experiments, bacterial cultures were exposed to netilmicin (Schering Laboratories, Bloomfield, N.J.) during a 28-h treatment period. The experimental design was based on delivery of the same total daily amount of netilmicin in each of three different dosage regimens: (i) a 60-min infusion every 24 h \([q24h]\), (ii) 60-min infusions containing one-third of the dose of regimen (i) \([q8h]\), or (iii) a continuous infusion \((CI)\). The nominal peak concentrations of netilmicin in the central compartment were 24 μg/ml for the \(q24h\) regimen, 8 μg/ml for the \(q8h\) regimen, and 3.7 μg/ml after the steady state was reached during the CI. These three regimens were tested against all six strains that were used in this study.

In addition to the above experiments, the three nonpseudomonal strains were also exposed to only one-eighth of the above daily dose over 28 h. A \(q8h\) regimen of three 60-min infusions per day was administered, each attaining a nominal peak concentration of 1 μg/ml (low-dose \(q8h\) regimen).

The drug was administered into the central compartment either by a syringe infusion pump (ASSA; Autosyringe, Hooksett, N.H.) during the 60-min infusions or by direct addition of the drug to the diluent reservoir for the CI experiments.

Bacteria. Six strains were used in this study. The respective netilmicin MICs and MBCs for these strains, established

FIG. 2. Netilmicin concentrations in the central (solid line) and peripheral (broken line) compartments during the administration of the same daily dose given either in one or in three 60-min infusions. Standard deviations of fourfold replications are shown.
by the broth microdilution technique (11), were as follows: *P. aeruginosa* ATCC 27853, 4 and 8 µg/ml; *P. aeruginosa* A-10, (clinical isolate) 8 and 16 µg/ml; *P. aeruginosa* 14974, (clinical isolate) 4 and 8 µg/ml; *E. coli* ATCC 25922, 1 and 2 µg/ml; *K. pneumoniae* ATCC 13883, 0.125 and 0.125 µg/ml; and *S. aureus* ATCC 29213, 0.125 and 0.25 µg/ml. The bacteria were stored in liquid nitrogen. Before use they were thawed and preincubated for 2 to 6 h to yield the desired inoculum. Subsequently, 0.4 ml of this bacterial suspension was injected into the peripheral compartments and incubated for 2 h before antibiotic was administered. This provided exponentially growing cultures with a geometric mean of $1.2 \times 10^7$ CFU/ml at the beginning of the drug infusions (range of 47 experiments, $1.7 \times 10^5$ to $1.4 \times 10^7$ CFU/ml).

Quantification of bacterial growth and killing. All three drug regimens were tested in duplicate against six bacterial strains. During each experiment, 0.3-ml samples were drawn 12 to 14 times over a 28-h period. Bacteria were counted by making 10-fold dilutions of the samples with sterile, chilled 0.9% NaCl solution and then plating 20 µl in triplicate on Mueller-Hinton agar. In addition, 100 µl of each sample was filtered through a 0.45-µm-pore-size filter (HA; 47-mm diameter; Millipore Corp., Bedford, Mass.) and cultured on agar. Plates and filters were read after sufficient incubation time to develop colonies at 37°C. For bacterial population analysis, Mueller-Hinton agar plates with netilmicin concentrations of 4, 8, and 16 µg/ml were used.

Susceptibility testing. MICs were measured in triplicate by the agar dilution technique (27) and by the broth microdilution method with inocula of both $10^9$ (11) and $10^8$ CFU/ml. MBCs were determined as $>99.9\%$ killing in a subculture volume of 10 µl. For the agar dilution technique (27), the desired inocula ($10^8$ CFU) were spotted in duplicate on each agar plate in volumes of 10 µl.

Postexposure MICs were measured by two agar dilution methods. When cultures were turbid, samples were drawn from the peripheral compartment, diluted appropriately, and spotted directly on netilmicin agar plates. When cultures were clear, samples were subcultured on Mueller-Hinton agar plates, and four to five colonies were again subcultured in supplemented Mueller-Hinton broth to yield logarithmically growing cultures which were then spotted on netilmicin agar plates. The two methods were compared by duplicate analysis of 18 samples. The bacterial concentration was adjusted by optical density measurement. All actual inocula were counted to confirm the desired number of CFU per spot ($5 \times 10^7$ to $5 \times 10^8$). Pre- and postexposure samples were spotted side by side on the same plates.

Data analysis. Geometric means were used for averaging bacterial populations determined in multiple experiments. If no CFU were detected per 100 µl in a given sample, a value of 5 CFU/ml was arbitrarily used for the geometric mean calculation. Doubling times during exponential growth were determined by linear regression analysis (24). Exponential regrowth occurred in some experiments during q24h and q8h regimens after an initial bactericidal effect. Analysis of this regrowth included data from the sixth hour up to the time bacterial counts reached $10^8$ CFU/ml. Analysis of variance was used to evaluate the dose response in terms of minimal bactericidal concentrations achieved during each of the three different regimens (24). The sign test of Dixon and Mood was used to compare the regimen response of the nonpseudomonal strains in terms of CFUs measured between 2 and 12 h (22). Emergence of resistant subpopulations due to netilmicin exposure was analyzed by logarithmic transforma-

mation of the CFU data followed by Student’s unpaired t test.

RESULTS

Model pharmacokinetics. The model exhibited linear two-compartment pharmacokinetics as shown in Fig. 2 for the q8h and q24h regimens. The netilmicin concentrations achieved in the central compartment were reproducible during replication of the same regimen (coefficient of variation, 5%). Concentrations in the peripheral compartments had an average coefficient of variation of 12% (four determinations at each of 15 time points).

The peak concentration in the peripheral chambers after a 60-min infusion lagged behind the central compartment by 1 h. The actual peak netilmicin concentration in the peripheral compartment as a proportion of that in the central compartment increased from 52 to 67 and 71% over the first three doses, reflecting the increasing residual concentrations in the peripheral compartments. During the CI regimen, the netilmicin levels in the central compartment at 2, 4, and 8 h were 58, 76, and 86%, respectively, of the steady-state concentration of 3.7 µg/ml. Corresponding concentrations in the peripheral compartments were 27, 62, and 89% of the steady-state concentration.

Regimen response of *P. aeruginosa*. Administering the same total daily dose by different regimens resulted in marked differences in the initial bactericidal effect with all *P. aeruginosa* cultures. However, bacterial regrowth to final densities of more than $3 \times 10^9$ CFU/ml occurred in all experiments despite the fact that all drug regimens were provided during the full 28-h period. The antibiotic was most effective, in terms of the lowest number of CFUs achieved, when administered in one 60-min infusion per day as shown in Fig. 3 for the A-10 strain. Similar results were found for the other two *P. aeruginosa* strains tested (Table 1).

Little or no bactericidal effect (less than 10-fold reduction of CFUs) was observed during exposure of the three strains to continuous infusion of netilmicin (Table 1). In contrast, bacterial numbers decreased by 2 to 5 orders of magnitude when the daily dose was given in one or three 60-min infusions. After this initial bactericidal effect, all cultures

![FIG. 3. Antibacterial effect of netilmicin on *P. aeruginosa* A-10.](http://aac.asm.org/)
TABLE 1. Effect of three netilmicin dosage regimens on initial killing and subsequent regrowth of three strains of *P. aeruginosa*

<table>
<thead>
<tr>
<th><em>P. aeruginosa</em> strain</th>
<th>Minimum bacterial concn achieved (log$_{10}$ CFU/ml) by regimen$^a$</th>
<th>Time (h) needed to return to initial bacterial concn after regimen$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CI</td>
<td>q8h</td>
</tr>
<tr>
<td>27853</td>
<td>5.7 ± 0.2</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>A-10</td>
<td>5.6 ± 0.1</td>
<td>2.0 ± 0.1</td>
</tr>
<tr>
<td>14974</td>
<td>5.3 ± 0.7</td>
<td>3.5 ± 0.1</td>
</tr>
</tbody>
</table>

$^a$ The same total daily dose was administered as a CI, as three 60-min infusions q8h, or as a single 60-min infusion q24h. All experiments were repeated in duplicate except for the CI of strain 27853, which was done in triplicate. Means ± standard deviations are given.

resumed growth, surpassing the bacterial population at the beginning of treatment within 21 h.

Significantly greater initial bacterial killing was achieved by the 60-min infusion regimens (q8h and q24h) compared with the CI regimen (Table 1; P < 0.01 for strains ATCC 27853 and A-10, and P < 0.05 for strain 14974, by analysis of variance). For the A-10 strain, the initial killing was significantly greater (P < 0.05) with the dose of 24 μg/ml (q24h) than with that of 8 μg/ml (q8h).

**Regimen response of *E. coli*, *K. pneumoniae*, and *S. aureus***.

In contrast to results with the three *P. aeruginosa* strains, the numbers of *E. coli* were consistently reduced to levels of <20 CFU/ml during treatment with each of the three regimens, and regrowth did not occur (Fig. 4). Similar effects were observed against *S. aureus* except for the q8h regimen, where in one of two experiments, bacterial numbers stayed above 20 CFU/ml (Fig. 4). Bacterial killing during treatment was analyzed by determining the time required to consistently reduce bacterial numbers to <100 CFU/ml without any subsequent regrowth. Based on this criterion, the q24h regimen was effective within less than 2 h for both *E. coli* and *S. aureus* as opposed to the q8h and CI regimens, where more than 12 h was required. For both organisms, the q24h regimen provided significantly lower CFUs between 2 and 12 h compared with the other two regimens (P < 0.02, sign test).

All regimens were very effective against *K. pneumoniae*. Only 40, 20, and <20 CFU/ml were measured for the CI, q8h, and q24h regimens, respectively, 2 h after treatment. At 4 h, each of these regimens had suppressed the cultures to <20 CFU/ml with no subsequent regrowth.

**MIC versus netilmicin concentration**. With all of these regimens, regrowth occurred with all strains of *P. aeruginosa*, but not with the other bacteria. However, netilmicin MICs for the *P. aeruginosa* strains (4 to 8 μg/ml) were higher than those for *E. coli* (1 μg/ml) and for *K. pneumoniae* and *S. aureus* (0.125 μg/ml). To determine whether the difference in regrowth reflected different ratios of MIC to netilmicin concentrations or indicated species-specific behavior during drug exposure, additional experiments were conducted with the three nonpseudomonads by using only one-eighth of the dose. The response to this low-dose q8h treatment of both *E. coli* and *S. aureus* was similar to the response of the *P. aeruginosa* strains exposed to the higher-dose q8h regimen (Fig. 5). However, even with the low-dose q8h treatment, *K. pneumoniae* was reduced to <20 CFU/ml by 6 h and did not grow thereafter.

**Effect of the first dose versus subsequent doses**. Administration of the second dose or subsequent doses during the intermittent dosing regimens (q8h and q24h) had minimal impact against *P. aeruginosa*, in contrast to the marked bactericidal effect observed after the initial dose. No killing occurred after the second dose despite lower bacterial concentrations than at the time of the first of the four q8h doses (Fig. 5). Identical results were obtained with *E. coli* and *S. aureus* during the low-dose q8h regimen. During the higher-dose q8h regimen, however, regrowing cultures of both *E. coli* and *S. aureus* did respond to the second dose and subsequent doses with further killing (Fig. 4).

Although no bactericidal effect was observed with subsequent intermittent doses against *P. aeruginosa* after 8 and 16 h, these doses did reduce the growth rate. The doubling times during growth in drug-free medium were 33, 26, and 37 min for strains ATCC 27853, A-10, and 14974, respectively. Regrowth during the q8h regimen yielded doubling times that were 139, 61, and 89% longer than the time for control growth of the respective strains. During the q24h regimen, the doubling times were increased by 42, 35, and 32% of control growth. A similar reduction of the growth rate was found when drug-free growth was compared with regrowth during the low-dose q8h regimen for *S. aureus* and *E. coli*. The doubling times increased by 86 and 72%, respectively.

**Selection of resistant populations**. The lack of persistent suppression of growth observed in all experiments with *P. aeruginosa* correlated with the selection of resistant subpopulations. Bacterial population analysis performed during the q24h regimen is shown in Fig. 6. Resistant subpopulations emerged with all strains after the first dose. This shift of the...
FIG. 5. Antibacterial effect of intermittent administration of netilmicin, given as 60-min infusions q8h. Three strains of P. aeruginosa (bottom panel) were exposed to netilmicin at nominal peak concentrations of 8 µg/ml. E. coli and S. aureus (top panel) were exposed to one-eighth of this amount of drug (peak concentrations of 1 µg/ml). Control growth curves (no drug) are given for all strains (solid symbols). Geometric means of duplicate experiments were plotted. For clarity, range is shown only for every 6 h.

bacterial population to variants resistant to netilmicin concentrations of 4 (\(P < 0.001\)) and 8 µg/ml (\(P < 0.01\)) was significant for all strains. The increase of netilmicin MICs above preexposure controls for the three strains as measured with the agar dilution technique is shown in Table 2. Similar observations were made during the low-dose q8h regimen for E. coli (fourfold MIC increase) and S. aureus (eightfold MIC increase).

Simultaneous comparisons of the two MIC methods (direct versus subculture [see above]) with 18 different samples served as the basis for Table 2. The MIC of netilmicin for three strains of P. aeruginosa is shown.

![Graph](http://aac.asm.org/)

**Figure 5.** Antibacterial effect of intermittent administration of netilmicin, given as 60-min infusions q8h. Three strains of *P. aeruginosa* (bottom panel) were exposed to netilmicin at nominal peak concentrations of 8 µg/ml. *E. coli* and *S. aureus* (top panel) were exposed to one-eighth of this amount of drug (peak concentrations of 1 µg/ml). Control growth curves (no drug) are given for all strains (solid symbols). Geometric means of duplicate experiments were plotted. For clarity, range is shown only for every 6 h.

**Figure 6.** Population analysis of *P. aeruginosa* ATCC 27853 during a q24h regimen. The first dose of netilmicin was administered at time 0. Colony counts of bacteria were performed on agar plates containing 0, 4, 8, and 16 µg of netilmicin per ml. The mean and range of duplicate experiments are shown.

**TABLE 2.** MIC of netilmicin for three strains of *P. aeruginosa*

<table>
<thead>
<tr>
<th><em>P. aeruginosa</em> strain</th>
<th>Before treatment</th>
<th>CI</th>
<th>q8h</th>
<th>q24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 27853</td>
<td>4</td>
<td>16</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>A-10</td>
<td>4</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>14974</td>
<td>4</td>
<td>16</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>

Determined with the agar dilution technique before and after a 28-h treatment either with a CI or with 60-min infusions administered q8h or q24h.
of *P. aeruginosa* demonstrated identical results with both techniques in 13 cases (72%). For the remaining five samples (28%), the MIC was reduced by a factor of 2 when determined with the standard method (subculture). Thus, in most experiments, increased resistance of pseudomonads during exposure to aminoglycosides was retained through at least two subculture steps.

**Reproducibility of bacterial response.** All 21 experiments were performed in duplicate and analyzed for reproducibility. The mean difference in bacterial concentrations in duplicate cultures exposed to the same dosage regimens was 0.48 ± 0.55 log₁₀ CFU/ml (± standard deviation).

**DISCUSSION**

The present paper describes an in vitro two-compartment model that mimics human pharmacokinetics. A sterile central compartment represents the systemic circulation. The peripheral chambers are compartments with a fast turnover, as represented experimentally in humans by small skin ulcer blisters (J. Blaser, Ph.D. thesis, Swiss Federal Institute of Technology, Zürich, 1981). Exponentially growing bacteria are exposed to changing concentrations of antibiotics (Fig. 1). This system simulates infection at an extravascular site and isolates the effects of antibiotic treatment in the absence of host defenses. Unlike other in vitro models (15, 16, 19, 23, 25, 29; B. Ledergerber, A. Hugentobler, J. Blaser, R. Lüthy, and M. Anliker Program Abstr. Inserci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 898, 1983), several organisms can be studied simultaneously over an extended time period against a given drug regimen, and the bacterial inocula are not diluted as the drug concentration decreases. The small volumes sampled (0.3 ml) to assay bacterial and drug concentrations do not significantly affect the inoculum (10 ml).

Once daily administration (q24h) and CI of the same total daily dose of an aminoglycoside were compared with the regimen which is clinically most frequently used (short infusions q8h). The daily dose of netilmicin given in vitro was chosen to closely mimic drug levels recommended for clinical use (18). Netilmicin was studied against the four pathogenic bacteria most frequently isolated from infected neutropenic patients (8).

Considerable differences in the effect of these regimens were found. Although no regimen completely sterilized the *P. aeruginosa* inocula, the once daily high peak netilmicin concentration (q24h) produced the most pronounced initial bactericidal activity. Thus, this regimen appears to offer the lowest inoculum for host defenses to clear. This regimen also yielded the steepest rate of bacterial killing and the shortest time period for eradication of *E. coli, K. pneumoniae*, and *S. aureus*.

These observations correlated with the ratios of the peak drug concentration to the MIC. *P. aeruginosa* eventually regrew at peak-to-MIC ratios of 6 or less (Fig. 3). Similarly, regrowth occurred during the low-dose q8h regimens when *E. coli* and *S. aureus* were exposed to peak-to-MIC ratios of 1 and 8, respectively (Fig. 5). In contrast to *S. aureus*, *K. pneumoniae* was eradicated during the low-dose q8h regimen. Both strains had the same MIC, but the MBC of *K. pneumoniae* was one dilution step lower. All regimens that provided peak-to-MIC ratios of >8 did reduce the inocula to <10⁵ CFU/ml without regrowth (Fig. 4).

Similar to the strains selected for this study, the majority of clinically isolated strains of *E. coli, K. pneumoniae*, and *S. aureus* are more sensitive to aminoglycosides than is *P. aeruginosa* (4, 5). This allows for the treatment of the former organisms with higher ratios of peak drug concentration to MIC and offers a better likelihood of a favorable clinical result.

Failure to eradicate the bacterial inocula despite exposure to antibiotic concentrations of up to eight times the MIC up to four times the MBC could reflect the pharmacokinetic parameters of the in vitro system that allows for only a short exposure to high drug concentrations. However, no killing occurred after the second dose and subsequent doses except when the peak-to-MIC ratio was high (8 for *E. coli*; 64 for *S. aureus* [Fig. 4]). It is unlikely that the oscillations in drug concentrations were primarily responsible for bacterial regrowth. Regrowth of *P. aeruginosa* also has been reported during constant exposure to inhibitory concentrations of aminoglycosides (B. Ledergerber, J. Blaser, and R. Lüthy, Programmed Abstr. Mediterranean Cong. Chemother. 3rd, Dubrovnik, Yugoslavia, abstr. no. 455, 1982).

The difference in the inoculum size used in the in vitro system (10 ml of 10⁶ CFU/ml) and the MIC determinations (10⁴ CFU per spot with the agar dilution and 0.1 ml of 10⁶ CFU/ml with the broth microdilution technique) offers an explanation for the fact that the MICs were not directly predictive of the response in the model. When MIC measurements were performed with the broth microdilution technique with the same absolute inoculum as in the model, the MICs and MBCs were increased by a factor of 4 (*P. aeruginosa* strains, *E. coli*) or more (*S. aureus, K. pneumoniae*). Woolfrey et al. (28) have compared micro- and macromethod MICs of 650 *P. aeruginosa* strains. The higher absolute inocula used with the macromethod might explain their findings of MICs two to four times higher than with the broth microdilution and agar dilution techniques.

A striking difference was observed between the effects of the first and second doses in all cultures in which the treatment failed. In contrast to the marked bactericidal effect of the initial dose, no (q8h) or only very limited (q24h) killing was seen after the second dose (Fig. 3, 5, 6). Measurements of PO₂ in the culture chambers showed that anaerobic conditions were present at the very high bacterial densities after 24 h. Under such conditions the activity of aminoglycosides is very limited (21). However, aerobic conditions and unchanged pH prevailed after 8 h, and no killing occurred after the second dose with the *P. aeruginosa* strains and during the low-dose q8h regimen with *E. coli* and *S. aureus*.

These observations might be explained in part by the results of the bacterial population analysis. The exposure of bacteria after the first dose of netilmicin induced the selection of resistant subpopulations (Fig. 6). These subpopulations either were present in the original inoculum and became more dominant after the more sensitive bacteria were killed or they emerged rapidly as part of an adaptation process. The regrowing strains displayed a two- to eightfold increase in MIC, generally corresponding to the peak concentrations of the respective regimens.

The selection of aminoglycoside-resistant variants and the lack of significant additional killing after 6 h despite additional drug infusions have been reported previously for gentamicin and *P. aeruginosa* in vitro (23rd ICAAC, abstr. no. 898) and in vivo (12, 14). In part, this phenomenon might explain both the failure of aminoglycoside monotherapies in clinical infections with *Pseudomonas* species and other gram-negative rods (2, 17) and the poor correlation of the results of in vitro testing of aminoglycosides with clinical outcomes (9, 10).
In Vitro Model for Netilmicin Dosing

The search for optimal dosage regimens of aminoglycosides against P. aeruginosa has been addressed in several in vivo models. In one study, the response of guinea pigs with acute pneumonia to once daily dosing was better than with CI of the same daily dose, although chronic pneumonia in rats and endocarditis in rabbits responded similarly to both regimens (20). In a thigh injection model in granulocytic mice, gentamicin was more effective when the same total dose was administered at 3-h rather than 1-h intervals (13).

The data presented here suggest that during monotherapy, a single daily dose of netilmicin provides a steeper and more pronounced reduction of a bacterial inoculum than more frequent drug administration. Powell et al. (20) demonstrated that infrequent, high peak serum concentrations might be less toxic than more frequent dosing to achieve lower peak serum levels. In their study, tobramycin was administered either once daily or continuously for 10 days to 52 patients with cystic fibrosis, with no adverse effects on hearing or creatinine clearance despite peak serum concentrations of 40 μg/ml. Thus, the concept of administering aminoglycosides q24h might be favorable in terms of efficacy and toxicity.

This pharmacokinetic model is an efficient tool for the in vitro study of bacterial response to antibiotic therapy. The feature of fluctuating drug concentrations similar to in vivo pharmacokinetics allows for the design of optimal antibiotic dosage regimens. Conclusions derived from such in vitro studies offer a rational basis for in vivo testing. In addition, in vitro models avoid the problem of the significantly faster drug elimination found in laboratory animals compared with human pharmacokinetics (14, 20).

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