Aminoglycosides are often used to treat severe infections with gram-positive organisms. Previous studies have shown concentration-dependent killing by aminoglycosides of gram-negative bacteria, but limited data are available for gram-positive bacteria. We compared the in vitro pharmacodynamics of gentamicin against \textit{Staphylococcus aureus} and \textit{Pseudomonas aeruginosa}. Five \textit{S. aureus} strains were examined (ATCC 29213 and four clinical isolates). Time-kill studies (TKS) in duplicate (baseline inocula of $10^7$ CFU/ml) were conducted to evaluate bacterial killing in relation to increasing gentamicin concentrations (0 to 16 times the MIC). Serial samples were obtained over 24 h to quantify bacterial burden. Similar TKS with \textit{P. aeruginosa} ATCC 27853 were conducted, and the time courses of the all bacterial strains were mathematically modeled for quantitative comparison. A dose fractionation study (using identical daily doses of gentamicin) in an in vitro hollow-fiber infection model (HFIM) over 5 days was subsequently used for data validation for the two ATCC strains. Model fits to the data were satisfactory; $r^2$ values for the \textit{S. aureus} and \textit{P. aeruginosa} ATCC strains were 0.915 and 0.956, respectively. Gentamicin was found to have a partially concentration-dependent killing effect against \textit{S. aureus}; concentrations beyond four to 8 times the MIC did not result in significantly faster bacterial killing. In contrast, a concentration-dependent profile was demonstrated in suppressing \textit{P. aeruginosa} regrowth after initial decline in bacterial burden. In HFIM, thrice-daily gentamicin dosing appeared to be superior to once-daily dosing for \textit{S. aureus}, but they were similar for \textit{P. aeruginosa}. Different killing profiles of gentamicin were demonstrated against \textit{S. aureus} and \textit{P. aeruginosa}. These results may guide optimal dosing strategies of gentamicin in \textit{S. aureus} infections and warrant further investigations.

Aminoglycosides (e.g., gentamicin) are often used clinically in combination with other antimicrobial agents such as beta-lactams or glycopeptides for the treatment of serious infections with gram-negative and gram-positive organisms. Previous studies have repeatedly demonstrated a concentration-dependent killing effect of aminoglycosides against gram-negative bacteria (5, 6, 14, 25); optimal patient outcomes and suppression of resistance emergence are associated with peak concentration (maximum concentration of drug in serum [$C_{\text{max}}$/MIC] ratio (3, 13, 15) or area under the concentration-time curve (AUC)/MIC ratio (16). There is also strong evidence suggesting that the first dose of an aminoglycoside is the most important in the course of therapy. Adaptive resistance is a phenomenon in which bacteria exhibit down-regulation of drug uptake upon frequent and repeated exposures to antimicrobial agents (26). Consequently, the first dose of aminoglycoside has the most bactericidal effect on the bacterial population. It has also been reported that attainment of a pharmacodynamic target ($C_{\text{max}}$/MIC $\geq$ 10) within 48 h of therapy is associated with an early therapeutic response (12). Since the likelihood of aminoglycoside-induced nephrotoxicity is believed to be dependent on the cumulative drug exposure and/or concentration above a certain threshold (8, 17, 20, 24), achieving a pharmacodynamic target early may shorten the duration of therapy and thus reduce the likelihood of drug-induced adverse effects. Consequently, once-daily (or extended-interval) administration of aminoglycosides has been widely adopted in many hospitals in the United States (4).

While the approach is intuitive and consistent with pharmacodynamic principles, limited data are available to describe the pharmacodynamic activity of aminoglycosides against gram-positive bacteria (e.g., \textit{Staphylococcus aureus}, viridans group streptococci, and \textit{Enterococcus} spp.). Questions remain if the same dosing strategy should be used for severe infection with gram-positive bacteria (e.g., endocarditis), and evaluation of bacterial killing with various aminoglycoside exposures is essential to optimize dosing strategies in the clinical setting. We explored the impact of increasing gentamicin concentrations and various dosing regimens on the drug’s activity against different bacteria. The objective of the study was to compare the in vitro pharmacodynamics of gentamicin against \textit{S. aureus} and \textit{Pseudomonas aeruginosa}.

(This study was presented in part at the Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., December 16 to 19, 2005.)

**MATERIALS AND METHODS**

**Antimicrobial agent.** Gentamicin powder was purchased from Sigma (St. Louis, MO). Stock solutions of 1,024 mg/liter in sterile water were prepared, aliquoted, and stored at $-70^\circ$C. Prior to each susceptibility test, an aliquot of the drug was thawed and diluted to the desired concentrations with cation-adjusted Mueller-Hinton II broth (Ca-MHB) (BBL, Sparks, MD).

**Microorganisms.** Five strains of \textit{S. aureus} were examined. Standard wild-type strain ATCC 29213 (American Type Culture Collection, Manassas, VA) and four clinical isolates (two oxacillin susceptible [strains 55 and 60] and two oxacillin resistant [strains 25 and 62]) were used. All clinical isolates of \textit{S. aureus} used were wild type and were found to be clonally unrelated, as determined by
randomly amplified polymorphic DNA testing (19). P. aeruginosa ATCC 27853 was used for comparison. The bacteria were stored at −70°C in Protect (Key Scientific Products, Round Rock, TX) storage vials. Fresh isolates were subcultured twice on 5% blood agar plates (Hardy Diagnostics, Santa Maria, CA) for 24 h at 35°C prior to each experiment.

Susceptibility studies and mutation frequencies. Gentamicin MICs/minimum bactericidal concentrations (MBCs) were determined for all bacterial strains in Ca-MHB using a modified broth macrodilution method, as described by CLSI (formerly NCCLS) (18). The final concentration of bacteria in each broth macrodilution tube was approximately 5 × 10⁸ CFU/ml of Ca-MHB. Serial twofold dilutions of drugs were used. The MIC was defined as the lowest concentration of drug that resulted in no visible growth after 24 h (instead of 16 to 18 h, as recommended by CLSI) of incubation at 35°C in ambient air. Samples (50 μl) from clear tubes and the cloudy tube with the highest drug concentration were plated on Mueller-Hinton agar (MHA) plates (Hardy Diagnostics, Santa Maria, CA). The MBC was defined as the lowest concentration of drug that resulted in ≥99.9% killing of the initial inoculum. The drug carryover effect was assessed by visual inspection of the distribution of colonies on medium plates. The studies were conducted in duplicate and repeated at least once on a separate day. Mutation frequency of resistance for each isolate was determined by plating approximately 1 × 10⁷ CFU/ml (200 μl) of bacteria on MHA plates with and without gentamicin supplementation at 3 times the MIC. Since susceptibility testing was performed in twofold dilutions and one tube (2× concentration) difference is commonly accepted as reasonable interday variation, quantitative cultures on drug-supplemented medium plates (at 3 times the MIC) would allow reliable detection of bacterial subpopulations with reduced susceptibility. The medium plates were incubated at 35°C for up to 24 (total population) and 72 h (subpopulations with reduced susceptibility), and then bacterial density from each sample was estimated as described below.

Time-kill studies. Time-kill studies were conducted in duplicate on separate days with different and escalating gentamicin concentrations. Six clinically achievable concentrations of gentamicin were used, normalized to 0 (control), 0.5, 1, 2, 4, 8, and 16 times the MIC. An overnight culture of the isolate was diluted 10-fold with prewarmed Ca-MHB and incubated further at 35°C until reaching late-exponential growth. The bacterial suspension was diluted with Ca-MHB accordingly based on absorbance at 630 nm; 15 ml of the suspension was transferred to 50-ml sterile conical flasks, each containing 1 ml of a drug solution at 16 times the target concentration. The final concentration of the bacterial suspension in each flask was approximately 1 × 10⁸ CFU/ml. The high inoculum used was to simulate the bacterial load in severe infection and to allow a resistant subpopulation(s) to be present at baseline. The experiment was conducted for 24 h in a shaker water bath set at 35°C.

Serial samples were obtained from each flask at baseline (placebo only) and at 2, 4, 8, 12, and 24 h to characterize the effect of various gentamicin exposure levels to the total bacterial population. Prior to culturing the bacteria quantitatively, the bacterial samples (0.5 ml) were centrifuged at 10,000 × g for 15 min and reconstituted with sterile normal saline to their original volumes in order to minimize the drug carryover effect. Total bacterial populations were quantified by spiral plating 10-fold serial dilutions of the samples (50 μl) onto MHA plates. The medium plates were incubated in a humidified incubator (35°C) for 24 to 72 h and the zones of inhibition were measured. The assay was linear (correlation coefficient of 0.99) using zone diameter versus the log of the standard drug concentration. The intraday and interday coefficients of variation for all standards were <4% and <6%, respectively.

Microbiologic response. Serial samples were also obtained at baseline; at 4, 8, and 24 h (predose and 4 and 24 h thereafter) in duplicate from each hollow-fiber system, for quantitative culture to define the effect of various drug exposures on the bacterial population. The samples (0.5 ml) to quantify the bacterial population were processed as described above.

RESULTS

Susceptibilities and mutation frequencies. The susceptibilities of the bacterial isolates to gentamicin were as shown in Table 1. Baseline resistant subpopulations were detected in all isolates. The mutation frequency of gentamicin resistance (more than 3 times the MIC) ranged from 1 in 3 × 10⁴ to 1 in 4 × 10⁵.

Time-kill studies. The killing profiles of gentamicin against S. aureus ATCC 29213 are as shown in Fig. 1A. A consistent trend was apparent for all five S. aureus isolates (see the supplemental data). Overall, the bactericidal activity appeared to be concentration dependent, as gentamicin concentration was increased from 0.5 to 4 times the MIC. However, the rate of killing seemed to plateau at concentrations beyond 4 to 8 times the MIC. These observations were in direct contrast to those observed with P. aeruginosa (Fig. 1B). A rapid reduction in
bacterial burden was seen within 2 h of gentamicin exposure (all concentrations), which was followed by regrowth. With increasing gentamicin concentrations, a concentration-dependent trend was observed with respect to the suppression of regrowth, consistent with previous observations (5, 6).

Pharmacodynamic modeling. The model fits to the data were satisfactory. The $r^2$ for *S. aureus* ATCC 29213, 55, 60, 25, and 62 and *P. aeruginosa* ATCC 27853 were 0.915, 0.946, 0.942, 0.942, 0.900, and 0.956, respectively (Fig. 2 [ATCC strains only; for others see the supplemental data]). The final model parameter estimates are as shown in Table 1, and the relationships between gentamicin concentration and bactericidal activity are as shown in Fig. 3 (ATCC strains only; for others see the supplemental data). Against *S. aureus*, killing of the predomi-

<table>
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<tr>
<th>Strain</th>
<th>MIC/MBC (µg/ml)</th>
<th>$K_g$ (h$^{-1}$)</th>
<th>$N_{max}$ (10⁶ CFU/ml)</th>
<th>$K_k$ (h$^{-1}$)</th>
<th>$C_{50k}$ (mg/liter)</th>
<th>$H$</th>
<th>$\beta$</th>
<th>$\tau$ (liters/mg · h)</th>
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<td></td>
<td></td>
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**FIG. 1.** Time-kill studies of gentamicin (as multiples of MIC) against *S. aureus* ATCC 29213 (A) and *P. aeruginosa* ATCC 27853 (B). Data are presented as means ± standard deviations based on duplicate experiments performed on different days.
nant bacterial population was the prominent feature observed (with minimal adaptation resulting in regrowth); the bactericidal activity observed was concentration dependent at low concentrations (less than 4 times the MIC), and further increase in killing activity became less substantial when the concentration was beyond 4 to 8 times the MIC (Fig. 3A). On the other hand, our modeling analysis revealed that maximal killing of the predominant (susceptible) \textit{P. aeruginosa} population was readily achieved with all the gentamicin concentrations used, as reflected in a rapid decline in bacterial burden within 2 h of exposure in the time-kill studies (Fig. 1B). However, the prominent feature observed was the concentration-dependent relationship with respect to the most resistant subpopulation present at baseline (full adaptation) (Fig. 3B), leading to the differential propensity of increasing gentamicin concentrations in suppressing regrowth over time. The values of the sigmoidicity constants ($H$) between \textit{S. aureus} and \textit{P. aeruginosa} were also noted to be dissimilar, partially explaining the difference in their concentration-killing profiles.

**Pharmacokinetic validation in hollow-fiber infection models.** All simulated gentamicin exposures were satisfactory; the $r^2$ values for once- and three-times-daily dosing were 0.962 and 0.989, respectively (data not shown).

**Microbiologic responses in hollow-fiber infection models.** The effect of different concentration-time profiles of gentamicin on \textit{S. aureus} ATCC 29213 and \textit{P. aeruginosa} ATCC 27853 were as shown in Fig. 4. Against \textit{S. aureus}, gentamicin dosing given 3 times daily appeared to be more bactericidal compared to once-daily administration, using identical daily doses (Fig. 4A). Both dosing regimens achieved substantial killing (approximately 5-log kill) at 24 h, but regrowth was apparent with repeated dosing over the next 4 days for the once-daily dosing regimen. On the other hand, sustained bacterial suppression over 5 days was observed with the 3-times-daily dosing regimen. This was in contrast to data for \textit{P. aeruginosa}, in which the dosing schedule did not appear to have a significant impact on the killing activity of gentamicin. As long as the daily dose remained identical, the time courses of bacterial burden over 5 days were similar.
regardless whether the entire daily dose was given at once or over three doses (Fig. 4B).

DISCUSSION

Aminoglycoside pharmacodynamics has revolved primarily around the gram-negative bacteria. Studies have demonstrated positive outcomes utilizing an extended dosing interval of aminoglycosides in infections with gram-negative organisms (13, 20). However, limited clinical experience with infections with gram-positive organisms is available (21), and aminoglycoside use has been mostly based on theory. In this study we strived to improve our understanding of their pharmacodynamic properties against gram-positive bacteria, specifically, S. aureus. Therefore, similar to the earlier studies of efficacy against gram-negative bacteria (5, 6), investigations with monotherapy of gentamicin were undertaken. Once the pharmacodynamic properties of the aminoglycosides are well understood, more clinically relevant studies with various antimicrobial agent combinations could be performed subsequently to improve patient care.

Our data revealed that the killing profiles of gentamicin against S. aureus and P. aeruginosa were different. First, in time-kill studies, the killing profiles of gentamicin against different strains of S. aureus were comparable but different from that observed with P. aeruginosa. The often-cited concentration-dependent killing was observed only in P. aeruginosa, not in S. aureus, over the (clinically relevant) concentration range examined. In addition to comparing the killing profiles against different bacteria qualitatively (visually), we modeled the experimental data mathematically in order to provide an objective and quantitative evaluation. The merits of our mathematical modeling approach over conventional pharmacodynamic modeling have been discussed previously (22). While the adherence of bacteria to the surface of the conical flasks was not considered in the evaluation of these in vitro experiments, the interpretation of the modeling results was consistent with the observations. We recognized that all S. aureus strains examined in the study had similar susceptibilities to gentamicin (0.5 to 1 μg/ml); using isolates with a broader range of susceptibility to gentamicin might have further enhanced the generalizability of our findings.

Since the drug concentrations in time-kill studies are static (constant over time), we felt that the clinical relevance of the results might not be very evident. Therefore, a hollow-fiber infection model (in which drug concentration fluctuates over time, resembling human elimination and repeated dosing) was used to provide further clinical insights of our findings. In the hollow-fiber infection models, the impact of dosing schedules of gentamicin on the bacteria was somewhat dramatic. Based on the modeling analysis, the killing activity of gentamicin against S. aureus began to plateau at 4 to 8 times the MIC (Fig. 3A). Consequently, high peak concentrations (beyond 8 times the MIC) resulting from once-daily administration would be unlikely to result in a substantial increase in bacterial killing. Coupled with a prolonged period in which drug concentration was negligible, once-daily dosing might not suppress/eradicate the bacteria as readily as a regimen with more frequent dosing. On the other hand, we found a concentration-dependent effect of gentamicin in suppressing regrowth of P. aeruginosa (up to at least 32 times the MIC, as shown in Fig. 3B). An enhanced bacterial killing rate against the resistant subpopulation was anticipated from high peak concentrations (approximately 12 times the MIC) associated with once-daily administration, which was offset by a prolonged period in which drug concentration was negligible (minimal killing). Therefore the overall extents of killing were comparable regardless of the dosing schedules (once versus thrice daily) as long as the total daily doses (drug exposure) remained identical. These observations from the hollow-fiber infection models were again consistent with our expectations and previous studies (2, 11). We verified the simulated gentamicin exposures were reasonable in the hollow-fiber infection models, but the mechanism of regrowth was not explicitly investigated. From the modeling perspective, regrowth after initial decline of the bacterial population was empirically attributed to adaptation in this study. Under antimicrobial selective pressure, a preexisting resistant subpopulation gradually replaced the entire population as the dominant clone over time; regrowth and the emergence of resistance were observed as a result. Furthermore, as long as the relationship between drug concentration and bactericidal activity is not saturable (concentration-dependent killing), the AUC/MIC ratio (primarily determined by the daily dose, regardless of dosing schedule) would be closely related to the cumulative killing over time. The postantibiotic effect of gentamicin was not specifically investigated in this study, in view of the doubtful clinical relevance reported in previous studies (9, 10).

In contrast to our data, in a recent in vitro study, the activ-
ities of gentamicin given once and 3 times daily against *S. aureus* in simulated endocardial vegetations were investigated in combination with daptomycin and vancomycin (23). The authors concluded that gentamicin given as a single large dose was superior to three smaller doses in combination with daptomycin or vancomycin. The experimental design of this study and our study differed in several ways, which prohibited direct comparison of the findings. Firstly, the bactericidal activity of gentamicin was examined in combination with other agents (daptomycin and vancomycin); therefore the pharmacodynamics of gentamicin might have been confounded by interaction (e.g., synergy or antagonism) with these agents used concurrently. Secondly, a simulated-infected-endocardial-vegetation model was used in the previous study (23); the observed killing in these simulated fibrin clots was (at least partially) dependent on the penetration (concentration achieved) of the antimicrobial agents inside the vegetations. Finally, the previous study was not strictly a dose fractionation study, as we have conducted. The total daily doses of gentamicin were not identical in the once-daily (simulating a human equivalent dose of 5 mg/kg of body weight every 24 h) and 3-times-daily (simulating a human equivalent dose of 1 mg/kg every 8 h) regimens. Therefore, the overall levels of killing by gentamicin might be expected to be different. In view of these differences, the pharmacodynamics of gentamicin against *S. aureus* might not have been interpreted concordantly by the two groups.

In conclusion, we found that gentamicin exhibited distinct killing profiles against *S. aureus* and *P. aeruginosa*. The well-accepted concentration-dependent bactericidal activity of the aminoglycosides may not be applicable against all bacteria. These results may guide optimal dosing strategies of gentamicin in staphylococcal infections and warrant further investigations.

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REFERENCES