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Once-Daily Aminoglycoside Dosing Assessed by MIC Reversion Time with *Pseudomonas aeruginosa*

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A novel, in vitro, pharmacodynamic comparison of single and divided daily dosing regimens of aminoglycosides is described. Experiments were conducted to evaluate the impact of gentamicin and tobramycin concentration on the time required for the MICs for five *Pseudomonas aeruginosa* strains to revert to their original values (MIC reversion time [MRT]) following single and multiple 2-h aminoglycoside exposures to 8 and 24 mg/liter. Single 2-h aminoglycoside exposures to 8 mg/liter produced culture MRTs (gentamicin, 21.5 ± 4.0 h; tobramycin, 22.3 ± 2.8 h) that were significantly ($P < 0.05$) shorter than those measured following identical exposures to 24 mg/liter (gentamicin, 28.9 ± 3.8 h; tobramycin, 26.8 ± 3.1 h). However, three sequential 2-h exposures to 8 mg/liter, one exposure every 8 h, produced MRTs following the third exposure (gentamicin, 68.1 ± 5.2 h; tobramycin, 77.8 ± 7.8 h) that were significantly longer ($P < 0.005$) than those determined following three 2-h exposures to 24 mg/liter, one exposure every 24 h (gentamicin, 36.1 ± 3.0 h; tobramycin, 34.5 ± 3.0 h). In addition, the once-daily exposure regimen to 24 mg/liter consistently produced cultures with significantly ($P < 0.005$) higher aminoglycoside concentration/MIC ratios compared with those for cultures reexposed to 8 mg/liter once every 8 h. These data support the concept of once-daily aminoglycoside dosing.

Aminoglycosides remain a cornerstone of therapy in the treatment of serious bacterial infections. They possess bactericidal activity against gram-negative bacilli, a limited tendency toward the development of resistance during therapy, and the lack of an inoculum effect (5). Pharmacodynamic research has shown that, following a single aminoglycoside exposure, these agents possess concentration-dependent bactericidal activity, a prolonged and concentration-dependent postantibiotic effect (PAE), and activity below the MIC, implying that they may be dosed once daily (12, 13, 15). Furthermore, the incidence of nephrotoxicity and ototoxicity with single-daily-dose aminoglycoside therapy is not increased, and in fact may be reduced, when compared with the incidence of nephrotoxicity and ototoxicity with the traditional divided daily dosing regimens (5, 12). However, the movement toward once-daily aminoglycoside dosing as an efficacious therapy must be tempered, because pharmacokinetic principles, in addition to pharmacodynamic concepts, should be considered (11, 13).

MICs and MBCs were the original in vitro measures of antimicrobial activity. The limitations of these static measures of antimicrobial activity have given way to more dynamic methods of describing the time course of antimicrobial activity. These methods include PAE, kill curves, subinhibitory MIC effects, adaptive resistance, and postantibiotic leukocyte enhancement (1, 4, 7, 11). Given the limitation of MICs as a single measure of antimicrobial activity, it was the intent of the study described here to measure MICs repeatedly at fixed intervals in order to assess susceptibility changes in bacterial cultures. Experiments were performed by comparing simulated traditional gentamicin and tobramycin dosing (2 mg/kg every 8 h [q8h], resulting in an approximate maximum concentration of drug in plasma [C_{max}] of 8 mg/liter) with once-daily dosing (6 mg/kg every 24 h [q24h], resulting in an approximate C_{max} of 24 mg/liter) on the basis of the time required for MICs to revert to their original values (MIC reversion time [MRT]) following single and multiple 2-h aminoglycoside exposures. In addition to the direct comparisons of MRTs after single and divided daily dosings that were made, the results also provided insight into changes in aminoglycoside concentration/MIC ratios for the *Pseudomonas aeruginosa* strains tested. The calculation and consideration of in vitro aminoglycoside concentration/MIC ratios are important, because C_{max}/MIC ratios have been correlated with clinical cure (3, 9). With respect to the present study, the reader should appreciate the limitations of conducting in vitro experiments with 2-h exposures at fixed aminoglycoside concentrations. Principal among these limitations is the knowledge that in vivo concentrations fluctuate constantly and that the half-lives of these agents are on the order of 2 to 3 h in patients with normal renal function.

Four clinical strains (F327, F443, F991, E1481) and one reference strain (ATCC 27853) of *P. aeruginosa* were used in the study. Isolates were frozen (−70°C) in skim milk and were transferred to blood agar plates monthly. At weekly intervals, isolates were subcultured onto fresh blood agar plates and were streaked for purity. The antimicrobial agents used were gentamicin (Schering Corp. Ltd., Pointe-Claire, Quebec, Canada) and tobramycin (Eli Lilly, Toronto, Ontario, Canada). Stock solutions of the antimicrobial agents were prepared from standard powders and were stored at −70°C. On the day of use, the concentrates were thawed and then diluted to achieve the desired concentrations. Cation (25 mg of $CaCl_2$ per liter, 12.5 mg of $MgSO_4$ per liter)-supplemented Mueller-Hinton broth (MHB; pH 7.2 to 7.4; Difco Laboratories, Detroit, Mich.) was used for all MIC determinations.

MIC determinations for each strain were made initially by the broth macrodilution method with the doubling dilutions described by the National Committee for Clinical Laboratory

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Standards (1990) (10). Following this, arithmetic antimicrobial dilutions were prepared in 1 ml of MHB, and MICs were determined to the nearest 0.1 mg/liter. The arithmetic MICs for each strain were determined in triplicate on separate occasions.

Experiments to determine the MRT were performed in triplicate for each strain following both single and multiple aminoglycoside exposures. An overnight broth culture incubated in a shaking water bath at 37°C was diluted 1:10 into fresh MHB and was allowed to regrow until the optical density of the culture at 580 nm approached 0.3 (Spectronic 1201 spectrophotometer; Milton Roy, Rochester, N.Y.). The resultant logarithmic-phase culture was then diluted 1:10 into MHB containing the desired concentration of aminoglycoside (8 or 24 mg/liter). Following a 2-h exposure, the aminoglycosides were removed by centrifugation. Test cultures and growth controls were centrifuged at 4,000 × *g* for 10 min, the supernatant above the bacterial pellet was decanted, and the pellet was resuspended in prewarmed (37°C) MHB to the initial volume. Two additional centrifugations were sufficient to reduce the antimicrobial concentrations to inactive levels. Residual antimicrobial agent controls ensured that the aminoglycoside concentrations present in cultures were reduced to inactive levels, as described previously (14). In addition TDX analysis (sensitivity, 0.1 mg of amikacin, gentamicin, netilmicin, and tobramycin per liter; Abbott Laboratories, Ltd., Mississauga, Ontario, Canada) was also used to confirm that the aminoglycoside concentrations were reduced to inactive levels. Cultures were allowed to regrow, with aliquots transferred to fresh MHB as necessary to maintain the cultures in the logarithmic phase.

The MICs determined in all single- and multiple-exposure MRT experiments were based on doubling dilutions by using multiples of the known arithmetically determined MIC. Cultures were considered to have reverted to their original susceptibility when the MIC for the culture was within 1 doubling dilution of its original value. MICs were determined every 4 h once a sufficient inoculum (approximately 5.5×10^5 CFU/ml) was present. Sufficient inocula were attainable at approximately 8 h following exposure to 8 mg/liter and 12 h following exposure to 24 mg/liter.

Multiple-exposure MRTs were determined by a modification of the single-exposure method described above. For 8 mg/liter, when a total of 8 h had passed from the time of the initial aminoglycoside exposure, the culture was diluted, if necessary, to approximately 10^6 to 10^7 CFU/ml and reexposed to 8 mg/liter. The entire procedure was repeated 8 h later. Multiple-exposure MRTs were determined following the third exposure. Similarly, three 2-hour aminoglycoside exposures to 24 mg/liter q24h were performed and the MRTs were determined.

Individual MRTs were taken as the midpoints of the 4-h intervals in which the MICs for the culture returned to within 1 doubling dilution of their original value. A minimum of 15 midpoint values was used to calculate each mean MRT and the standard deviation. Statistical analysis was performed by using *t* tests, with significance being established when P was <0.05.

The mean time for the MICs for the culture to fall below or to the aminoglycoside dosing concentration was defined as the midpoint of the 4-h interval in which the MIC fell below or to the aminoglycoside concentration used. In several instances, means and standard deviations were not calculated because one or more of the individual values did not exceed the aminoglycoside dosing concentration. Statistical analysis, when possible, was performed as described above for statistical analysis of the MRTs. The design of the study also allowed for the in vitro assessment of aminoglycoside concentration/MIC ratios. Just preceding each of the aminoglycoside exposures to 8 mg/liter (q8h) and 24 mg/liter (q24h), MICs were determined. These values were then divided by the appropriate dosing concentration to give aminoglycoside concentration/MIC ratios. Mean aminoglycoside concentration/MIC ratios and their standard deviations were calculated. Statistical analyses of the differences between aminoglycoside dosing concentrations and between the individual aminoglycosides themselves were performed by using *t* tests, with significance being established when P was <0.05. Standard analysis of variance calculations was used to check for differences between aminoglycoside reexposure groups within a particular aminoglycoside dosing regimen. If a significant difference was detected between groups, Tukey's multiple comparison test was then used to identify the specific location of the difference(s) between groups. Statistical significance was determined when P was <0.05.

TABLE 1. Mean MRTs and mean times for MICs to fall below or to the aminoglycoside dosing concentration following single 2-h aminoglycoside exposures in *P. aeruginosa*[a]

Aminoglycoside	Aminoglycoside dosing concn (mg/liter)	MRT (h)[b]	Mean time for MIC to fall below or to aminoglycoside dosing concn (h)[b]
Gentamicin	8	21.5 ± 4.0 (16–32)	12.4 ± 2.0 (8–16)
	24	28.9 ± 3.8 (24–40)	<12–16[c]
Tobramycin	8	22.3 ± 2.8 (16–28)	<8–8[c]
	24	26.8 ± 3.1 (20–32)	<12[c]

[a] Values are means ± standard deviations for all five strains tested. Determinations were repeated at least three times for each strain.
[b] Values in parentheses are ranges for all strains tested.
[c] Mean ± standard deviation was not calculable because some or all individual values did not exceed the aminoglycoside dosing concentration.

For the five *P. aeruginosa* strains tested, original, arithmetically determined MICs ranging from 2.5 to 3.6 mg/liter for gentamicin and 0.8 and 1.1 mg/liter for tobramycin were demonstrated.

MRTs are presented in Tables 1 and 2 for both gentamicin and tobramycin for all aminoglycoside concentrations and intervals tested. Following a single 2-h treatment with 8 mg/liter, MICs at 8, 12, and 24 h were four- to eightfold, four- to eightfold, and two- to fourfold greater, respectively, than the original MIC and did not return to their original values for between 16 and 32 h (gentamicin, 21.5 ± 4.0 h; tobramycin, 22.3 ± 2.8 h) (Table 1). Following a single 2-h treatment with 24 mg/liter, MICs at 12 and 24 h posttreatment were four- to eightfold and two- to fourfold greater, respectively, than the original MIC and did not return to their original values for 20 to 40 h (gentamicin, 28.9 ± 3.8 h; tobramycin, 26.8 ± 3.1 h). Multiple aminoglycoside exposures significantly increased the MRTs for cultures exposed to 8 mg/liter. Following triplicate 2-h treatments with 8 mg/liter q8h, MICs at 8, 12, and 24 h preceding the last treatment were 8- to 16-fold greater than the original MIC of both gentamicin and tobramycin and did not return to their original values for between 56 and 96 h (gentamicin, 68.1 ± 5.2 h; tobramycin, 77.8 ± 7.8 h). Following the triplicate exposure of cultures to 24 mg/liter for 2 h q24h, MICs at 12 and 24 h posttreatment were four- to eightfold and two- to fourfold greater, respectively, than the original values, with 28 to 40 h (gentamicin, 36.1 ± 3.0 h; tobramycin, 34.5 ± 3.0 h) required for MICs to revert to their original values.

TABLE 2. Mean MRTs and mean times for MICs to fall below or to aminoglycoside dosing concentration following multiple 2-h aminoglycoside exposures in *P. aeruginosa*[a]

Aminoglycoside	Aminoglycoside dosing concn (mg/liter)	MRT (h)[b]	Mean time for MIC to fall below or to aminoglycoside dosing concn (h)[b]
Gentamicin	8, 2 h, q8h, 3 times	68.1 ± 5.2 (56–80)	60.1 ± 7.1 (44–72)
	24, 2 h, q24h, 3 times	36.1 ± 3.0 (28–40)	15.1 ± 3.2 (12–24)
Tobramycin	8, 2 h, q8h, 3 times	77.8 ± 7.8 (64–96)	32.4 ± 3.3 (24–40)
	24, 2 h, q24h, 3 times	34.5 ± 3.0 (28–40)	<8[c]

[a] Values are means ± standard deviations for all five strains tested. Determinations were repeated at least three times with each strain.
[b] Values in parentheses are ranges for all strains tested.
[c] Mean ± standard deviation was not calculable because some or all individual values did not exceed the aminoglycoside dosing concentration.

A single exposure to 8 mg/liter produced significantly shorter MRTs than exposure to 24 mg/liter for gentamicin ($P < 0.01$) and tobramycin ($P < 0.05$). No significant differences were observed between gentamicin and tobramycin after single 2-h exposures to either 8 or 24 mg/liter. MRTs following multiple exposures to 24 mg/liter were significantly shorter than those following exposures to 8 mg/liter for both gentamicin ($P < 0.005$) and tobramycin ($P < 0.005$). Significant differences were seen between gentamicin and tobramycin in only one instance. MRTs following multiple exposures to tobramycin at 8 mg/liter were significantly longer ($P < 0.05$) than those for gentamicin.

Tables 1 and 2 show the mean times for the MICs for the cultures to fall below or to the aminoglycoside dosing concentration at which the MICs were calculable. Often for one or more strains, the aminoglycoside dosing concentration was not exceeded at the earliest MIC determination, and therefore means and standard deviations cannot be accurately provided. In these cases, only ranges are provided. Comparable data were obtained for multiple exposures to gentamicin at 8 and 24 mg/liter. The time required for the gentamicin concentration to fall below or to the aminoglycoside dosing concentration was significantly shorter ($P < 0.005$) for cultures exposed in triplicate to 24 mg/liter (q24h) than for cultures exposed in triplicate to 8 mg/liter (q24h). In addition, MICs for cultures exposed to gentamicin at 8 mg/liter in triplicate required significantly more time ($P < 0.005$) to fall below the aminoglycoside dosing concentration than the MICs for identical cultures exposed to tobramycin.

The once-daily regimen of exposure to 24 mg of aminoglycosides per liter consistently produced cultures for which the aminoglycoside concentration/MIC ratios were significantly higher ($P < 0.005$) compared with those for cultures re-exposed to 8 mg/liter q8h (Table 3). All tobramycin concentration/MIC ratios determined were significantly ($P < 0.001$) larger than the gentamicin concentration/MIC ratios measured over the same dosage interval at both 8 mg/liter (q8h) and 24 mg/liter (q24h). All first-exposure aminoglycoside concentration/MIC ratios were significantly ($P < 0.005$) greater than second-exposure aminoglycoside concentration/MIC ratios, regardless of the aminoglycoside or the regimen tested. In a comparison of second and third aminoglycoside exposures, only a single statistically significant difference was determined. Tobramycin exposed to 8 mg/liter as one exposure q8h had a significantly ($P < 0.01$) higher concentration/MIC ratio just preceding the second exposure compared with that measured just prior to the third exposure.

TABLE 3. Comparison of aminoglycoside concentration/MIC ratios on multiple gentamicin and tobramycin exposures at 8 and 24 mg/liter[a]

Aminoglycoside	Dose (mg/liter) and interval	Aminoglycoside concn/MIC ratio		
		First exposure	Second exposure	Third exposure
Gentamicin	8, q8h	2.5 ± 0.4	0.4 ± 0.3	0.2 ± 0.3
	24, q24h	7.5 ± 1.1	2.1 ± 0.3	1.9 ± 0.4
Tobramycin	8, q8h	8.9 ± 1.0	2.7 ± 0.2	1.0 ± 0.3
	24, q24h	26.7 ± 2.8	8.0 ± 1.0	6.2 ± 0.5

[a] Values are means ± standard deviations for all five strains tested. Determinations were repeated at least three times with each strain.

Despite the introduction of carbapenems, monobactams, and fluoroquinolones, aminoglycosides remain important antimicrobial agents in the therapeutic armamentarium. Aminoglycosides demonstrate rapid, concentration-dependent bactericidal activities, concentration-dependent PAEs, and activities at subinhibitory concentrations, suggesting that they may be used once daily. Recent work on the pharmacodynamics of aminoglycosides has also shown that the larger the aminoglycoside concentration/MIC ratio, the greater the rate and extent of bacterial killing (15). The rationale for using once-daily aminoglycoside dosing from a clinical efficacy point of view relates to the fact that the C_{max}/MIC ratio has been correlated to clinical cure (3, 8). Our study compared simulated traditional aminoglycoside dosing (2 mg/kg q8h, attaining an approximate C_{max} of 8 mg/liter) with once-daily aminoglycoside dosing (6 mg/kg q24h, attaining an approximate C_{max} of 24 mg/liter) with regard to the time required for the MICs for *P. aeruginosa* cultures to revert to their original values. In addition, the times required for the MICs for the cultures to fall below or to the aminoglycoside dosing concentration and the aminoglycoside concentration/MIC ratios were also noted.

The results of the present study demonstrated that MICs for *P. aeruginosa* cultures increase following single and multiple (q8h and q24h) exposures to gentamicin and tobramycin at 8 and 24 mg/liter. The MRTs following three 2-h exposures q24h (once-daily dosing regimen) were significantly ($P < 0.005$) shorter than the MRTs for cultures exposed to three 2-h exposures q8h (traditional dosing regimen) (Table 2). An explanation for this difference may lie in the observation that cultures exposed to 8 mg/liter q8h never returned to their initial susceptibilities between exposures, implying that subsequent exposures selected and maintained cultures for which the MICs were increasing. In comparison, cultures exposed to 24 mg/liter q24h returned to, or very close to, their initial susceptibilities between doses. Furthermore, the mean time for the MICs to fall below or to the aminoglycoside dosing concentration was significantly ($P < 0.005$) shorter for the once-daily aminoglycoside dosing regimen than for traditional dosing regimen (Table 2). Significantly ($P < 0.005$) higher

aminoglycoside concentration/MIC ratios with once-daily dosing compared with those with traditional dosing were also noted. These data lend support to the concept of once-daily aminoglycoside dosing.

In light of the data presented here, why are aminoglycosides clinically useful agents? It is our feeling that the results presented here must be considered within the context of clinical aminoglycoside usage. Almost invariably, aminoglycosides are administered in combination with one or more other agents, usually a beta-lactam, which may suppress or mask the development of this transient resistance. We are investigating this hypothesis.

In addition, our group has recently performed in vitro experiments detailing the PAE of *P. aeruginosa* following single and multiple aminoglycoside exposures (6). PAE is the recovery period or persistent suppression of bacterial growth following a short (1- or 2-h) antimicrobial exposure. It has been assumed that because post-PAE cultures resume logarithmic-phase growth, they have returned to their original, wild-type susceptibility (1). We found that repeated aminoglycoside exposures of *P. aeruginosa* at times well after the PAE interval was known to have passed (cultures exhibiting logarithmic-phase growth) produced significant reductions in bacterial killing and PAE, implying that post-PAE-phase cultures have not returned to their initial susceptibilities (6, 8). This suggests that the mechanism of the PAE with aminoglycosides against *P. aeruginosa* is independent of the selection of cultures with reduced susceptibilities. An adaptive resistance period lasting substantially longer than the PAE has been demonstrated following aminoglycoside exposure in *P. aeruginosa* (2). Our own initial studies detailing adaptive resistance (data not shown) have demonstrated a correlation between adaptive resistance and MRT, which we are continuing to investigate.

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