THE JOURNAL OF INFECTIOUS DISEASES • VOL. 145, NO. 4 • APRIL 1982
 0022-1899/82/4504-0020$00.75

Constant Infusions vs. Intermittent Doses of Gentamicin Against *Pseudomonas aeruginosa* in Vitro

Andreas U. Gerber, Peter Wiprächtiger, Ursula Stettler-Spichiger, and Gerhard Lebek

From the Department of Medicine and Institute of Medical Microbiology, University of Bern, Bern, Switzerland

Comparative studies were performed in vitro to test the advocated superiority of infusion over intermittent injection of aminoglycosides. *Pseudomonas aeruginosa* was exposed to constant and to continuously decreasing (simulating in vivo kinetics) concentrations of gentamicin. In comparing the effect with similar area-under-the-concentration-vs.-time curves, a substantial difference in killing and regrowth could not be demonstrated. Regrowth occurred only when the gentamicin concentration had continuously decreased below one fourth of the minimal inhibitory concentration for >2 hr. Exposure of *P. aeruginosa* to gentamicin for 30 min was followed by persistent suppression of bacterial regrowth for 1.4–1.9 hr. Thus, intermittent exposure of *P. aeruginosa* to gentamicin is as effective as constant exposure in vitro. The demonstrated persistent postantibiotic effect might cover in part the periods between intermittent doses of gentamicin in vivo as well as in vitro.

Septicemia caused by *Pseudomonas aeruginosa* is a major problem in leukopenic patients [1, 2]. The infection is usually treated with the combination of a β-lactam antibiotic and an aminoglycoside. Therapeutic failures with an aminoglycoside alone are numerous and often occur despite the in vitro demonstration of susceptibility by the causative pathogen [3, 4]. This discrepancy between in vitro susceptibility and clinical outcome could in part be explained by the fact that in routine sensitivity testing the organisms are exposed to a constant concentration of drug; in the clinical situation, however, drug levels are changing and during the interval between doses usually decrease below the MIC for the infecting organism. The small therapeutic index of the aminoglycosides often does not allow a substantial increase in dosing. In various clinical studies, an attempt was therefore made to improve the efficacy of aminoglycosides by administering these drugs as constant infusions rather than as intermittent injections [5–7]. However, no study so far has demonstrated a significant superiority of infusion over intermittent injection of aminoglycosides. Pertinent in vitro studies are lacking, although they would seem to be an important first approach to analyzing the effect of changing concentrations of drug on the isolated target.

In the present investigation, an in vitro model was used to compare the efficacy of constant vs. continuously decreasing concentrations of gentamicin against *P. aeruginosa*. Patterns of killing and regrowth of the test organisms were studied when the in vivo t½ of gentamicin was simulated in the model. Besides conventional inocula and log-phase cultures, large inocula and lag-phase organisms were also studied because infecting organisms can often reach far higher numbers in vivo than are usually studied in the test tube; it is probable that some lag-phase organisms are present even at the site of a virulent infection.

Received for publication February 17, 1981, and in revised form September 22, 1981.

Part of this study was presented at the First International Symposium of Infections in the Immunocompromised Host, held on June 1–5, 1980, in Veldhoven, the Netherlands.

This work was supported by funds from Beecham A.G., Bern, and Essex A.G., Lucerne, Switzerland.

Please address requests for reprints to Dr. Andreas U. Gerber, University of Bern, Clinic of Internal Medicine, Inselspital, 3010 Bern, Switzerland.

Materials and Methods

Organisms. *P. aeruginosa* strain no. ATCC 9721 (American Type Culture Collection, Rockville, Md.) and clinical isolate no. 14974 (Institute of Medical Microbiology, Bern, Switzerland) were studied. All experiments were performed with both strains.

Drug. Gentamicin sulfate was obtained from Essex A.G., Lucerne, Switzerland.

Media. Mueller-Hinton broth (Ca^{++}, 2.4 mg/liter; Mg^{++}, 1.95 mg/liter) was used. For a few

experiments, the broth was supplemented with Ca^{++} and Mg^{++} according to the procedure of Stratton and Reller [8]. Trypticase soy agar (Ca^{++}, 38.7 mg/liter; Mg^{++}, 17.0 mg/liter) was used for the cfu determinations and for semiquantitative population analyses on plates containing gentamicin in a concentration gradient (0–8 μg/ml). Agar plates with a higher concentration of divalent cations were used to reduce the carry-over effect of gentamicin activity for cfu determinations.

Inocula. Cultures that had been incubated for 24 hr were appropriately diluted whenever lag-phase organisms were studied. Logarithmic-phase organisms were obtained by diluting an overnight culture 1:100 in prewarmed broth and by further incubation at 37 C for 2 hr. This procedure resulted in $\sim 10^7$ viable organisms/ml.

Bacterial counts. Sterile 0.85% NaCl was used for serial dilutions of cultures as required. One-tenth milliliter of each dilution was plated on predried trypticase soy agar plates and, in some experiments, on plates with a gentamicin concentration gradient. The plates were inspected for growth after incubation for 24 hr at 37 C.

Gentamicin assay. The agar diffusion method using *Bacillus subtilis* strain no. ATCC 6051 (American Type Culture Collection) as the indicator organism was used. Standard curves were constructed with samples of known gentamicin concentration in medium identical to that of the unknown samples.

Determination of MIC, MBC, and minimal active concentration (MAC) of gentamicin. The MIC was determined repeatedly in broth [9]. The MBC was defined as a reduction of ≥3 log in the number of viable organisms at 18–24 hr of incubation. The MAC, defined as the minimal drug concentration resulting in a deviation from the growth curve, was determined for various inocula by constructing killing curves using 4-ml broth cultures.

Model for in vitro simulation of in vivo t½ of gentamicin. The model for in vitro simulation of the in vivo t½ of gentamicin (figure 1) was similar to the ones used by Grasso et al. [10] and Bergan et al. [11]. All cultures and the flasks with diluent were kept in a water bath at 37 C with shaking beginning 30 min before the experiment was begun. The experiment was started by addition of the drug into the test culture and by starting the peristaltic pump (Minipuls® II Gilson; Synmedic A.G., Zurich, Switzerland). The overflow was regulated by the pressure in the tightly stoppered test flask, thus keeping the volume of the test culture constant and yielding a continuous sample for the determination of cfu. The t½ of the drug (2 hr) was calculated by the volume of the test cultures (47–50 ml) and the inflow rate of the drug-free diluent according to the equation: $t½ = (\ln 2 \times \text{volume of test culture})/\text{inflow rate}$. The t½ was verified in each experiment by measuring the volumes of the test culture and the outflow. This procedure to calculate the drug kinetics in the system was verified using phenol red as the indicator. The *A* at 546 nm was measured in 0.1 N NaOH. An almost perfect correlation was found between the determined and the calculated levels of drug. All experiments were performed in two to four sets as shown in figure 1, using the same multichannel peristaltic pump. Thus, the cultures exposed to decreasing concentrations of gentamicin and those exposed to constant concentrations were always tested in parallel. They were diluted identically with the exception that the diluent of the constant-concentration cultures contained drug to

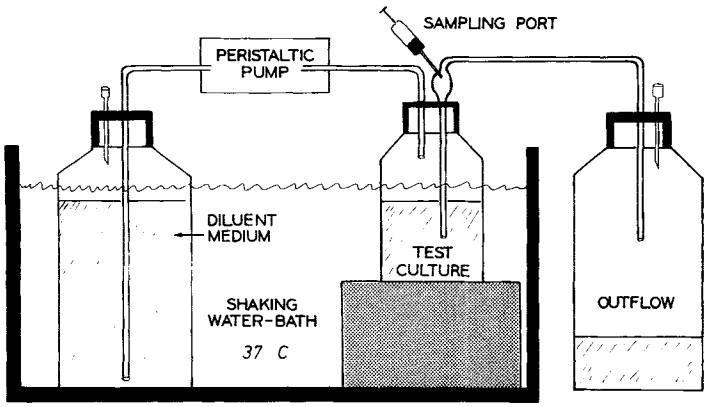

Figure 1. In vitro model used to simulate the in vitro kinetics of gentamicin against bacteria. Two to four cultures were tested in parallel.

keep the level in the test flask in a dynamic steady state. An inevitable drawback to this model is the dilution of the bacterial test culture, which in a 6-hr experiment was roughly eightfold.

Determination of persistent postantibiotic effect (PAE). The persistent PAE was determined under static conditions. After the organisms were exposed to gentamicin (at 37 C in a water bath without shaking), the drug was removed by adding 10 mg of sterile cellulose phosphate powder (Whatman, Maidstone, England) [12] suspended in 0.1 ml of Mueller-Hinton broth, which after vigorous shaking for 1 min in turn was removed by centrifugation at 100 *g* for 5 min. The organisms stayed in the supernatant. All cultures were checked for residual drug (using a highly gentamicin-susceptible clinical isolate of *Aerobacter*) and then diluted at least 10-fold into fresh prewarmed medium. The resulting subcultures (in triplicate) were incubated again at 37 C in a water bath without shaking and sampled hourly for determination of cfu. Control cultures were treated by the same procedure as the test cultures except that the cellulose phosphate powder was added simultaneously with the drug. In preliminary experiments the two *P. aeruginosa* strains grew in Mueller-Hinton broth from which gentamicin had been removed with cellulose phosphate powder as well as in normal Mueller-Hinton broth; no difference was observed when the growth rate of *P. aeruginosa* in normal Mueller-Hinton broth was compared with that in a mixture of 90% normal Mueller-Hinton broth and 10% Mueller-Hinton broth from which added gentamicin had been removed by the procedure described above. The PAE was quantitated as previously reported [13]. (*1*) The mean time (hr) required for the cfu count in the test subcultures (preexposed to gentamicin) to increase by 1 log above the count of cfu determined at the start of the test subcultures was measured. (*2*) The mean time (hr) required for the cfu count in the control subcultures to increase by 1 log above the count of cfu determined at the start of the control subcultures was measured. (*3*) The difference between the two values represents the time during which the drug affects bacterial growth after exposure (PAE time). In addition, the PAE time was calculated after an increase in cfu counts by 2 log in both test and control subcultures. A prerequisite to evaluating the PAE experiments was that the PAE time calculated after 2 log of regrowth in the subcultures was within 90%–110% of the PAE time calculated after 1 log of regrowth.

Calculations and statistical analysis of results. The area under the curve (AUC) of gentamicin concentration vs. time up to each sampling point was calculated by the trapezoidal rule. All cfu determinations in the kinetic model from test and control cultures were corrected for the dilution of the test organisms by the medium flow according to the equation: log cfu corrected = log cfu determined + (log 2 × [t/t½]), where t½ is the value calculated above and t is the time after starting the continuous dilution. This correction is based on the assumption that the outflushed bacteria would behave exactly as the bacteria remaining in the test flasks if by any means the outflushed bacteria could have been retained.

In a recent study by Murakawa et al. [14], cfu values in the kinetic model that they used were similarly corrected for outflushed bacteria.

The comparison of regression lines was performed by analysis of covariance [15].

Results

Effect of constant gentamicin concentrations. The MIC of gentamicin for both strains of *P. aeruginosa* was 0.4 μg/ml. The MAC for both strains was 0.1 μg/ml. MACs were identical for a small (10^3 cfu/ml) and a conventional ($10^{5.6}$ cfu/ml) inoculum; in contrast, MICs and MBCs were inoculum-dependent. When cultures of $>10^7$ cfu/ml were exposed to gentamicin in concentrations of up to 2 μg/ml (8 μg/ml in Ca^{++}- and Mg^{++}-supplemented broth), regrowth after the initial killing was common. Drug inactivation was excluded by comparative determinations of gentamicin activity in filtered (pore size, 0.45 μm) samples of cultures at the beginning and at the end of the experiment. It could easily be shown on plates with a gentamicin concentration gradient that a more resistant subpopulation was selected whenever such a large inoculum was used.

Supplementation of Mueller-Hinton broth with Ca^{++} and Mg^{++} increased the MICs, MACs, and MBCs of gentamicin for both strains of *P. aeruginosa* up to five to 10 times and shifted killing curves accordingly. Supplementation did not

otherwise alter the shape of the killing curves, nor did it suppress the selection of more gentamicin-resistant organisms.

Experiments performed under static conditions demonstrated that killing by gentamicin was similar for test organisms in the lag and logarithmic phases of growth. Killing started within the first 30 min of exposure to the drug and, up to 6 hr, was log-linear thereafter. At 0.2–2 μg of gentamicin/ml, the bactericidal effect and the killing velocity correlated with the drug concentration. These findings were of particular importance for evaluation of the results obtained in the kinetic model.

Effect of continuously decreasing gentamicin concentrations. Some killing curves obtained in the kinetic model are shown in figure 2. The gentamicin concentration in the two decreasing-concentration cultures was initially 0.4 and 0.8 μg/ml, respectively, and subsequently decreased with a t½ of 2 hr. The two constant-concentration cultures were exposed to 0.4 and 0.8 μg/ml, respectively, in a dynamic steady state. Dilution of all four cultures was started at 30 min after addition of the drug. In the two decreasing-concentration cultures, the drug concentration decreased to less than the MAC by 4.25 and 6.25 hr, respectively, after the drug was added.

Three features that were apparently not interrelated were consistently observed: (*1*) drug concentration- and time-dependent killing of *P. aeruginosa* up to 6 hr after addition of the drug (killing phase); (*2*) persistent suppression of bacterial regrowth at drug levels less than the MAC (postantibiotic phase); and (*3*) breakthrough growth in all cultures after 6–8 hr (regrowth phase).

Killing phase. At constant and at decreasing drug levels, the bactericidal effect of gentamicin up to 6 hr proved to be both concentration- and time-dependent. The individual points of killing curves from 1 to 6 hr were, therefore, mathematically transformed and plotted as the log of the percentage of surviving organisms vs. the log of

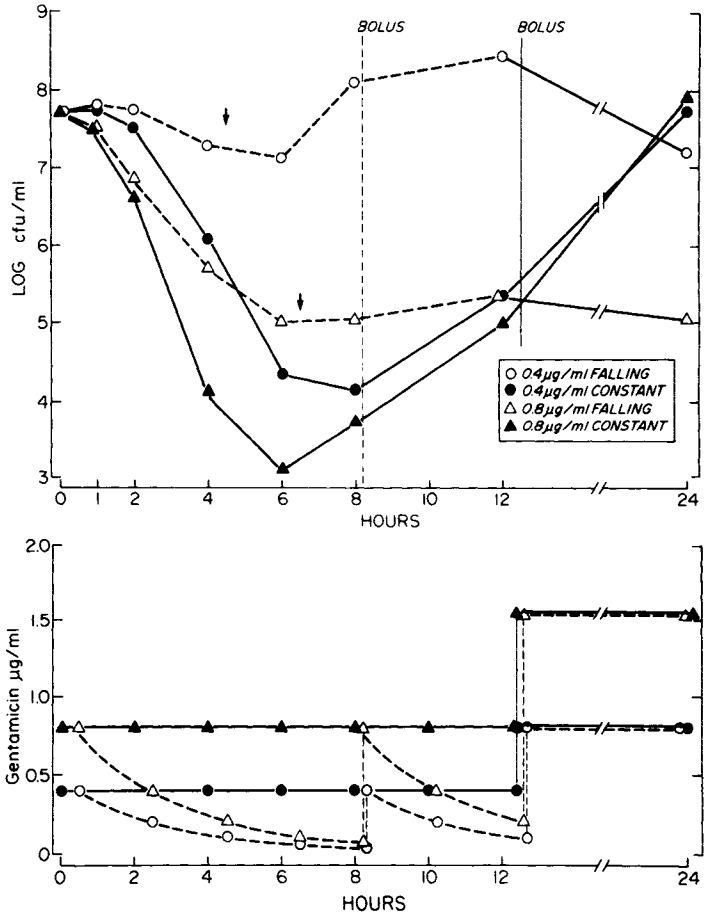

Figure 2. *Top*, killing curves of gentamicin against *Pseudomonas aeruginosa* strain no. ATCC 9721 as obtained in the kinetic model (figure 1) with unsupplemented Mueller-Hinton broth; *bottom*, calculated kinetics of gentamicin in continuously decreasing-concentration and constant-concentration cultures. The arrows indicate the time by which the gentamicin concentration decreased to less than the minimal active concentration (0.1 μg/ml). At 8 hr a second bolus was injected into the two decreasing-concentration cultures to increase the gentamicin concentration to the 0-hr level; at 12 hr the concentration was increased to twice the 0-hr value in all four cultures, and dilution was stopped. All cfu values are corrected for the dilution effect on the bacteria.

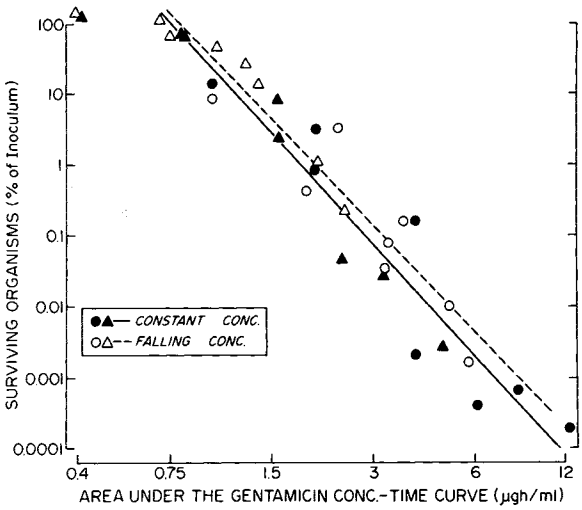

Figure 3. Effect of gentamicin on large inocula of *Pseudomonas aeruginosa* strain no. ATCC 9721 in the kinetic model (figure 1) with unsupplemented Mueller-Hinton broth. The results from figure 2 are indicated by triangles. Regression lines are calculated from data obtained with an area under the curve of gentamicin concentration vs. time equal to and greater than the minimal effective value (0.75 μg/ml).

the corresponding AUC of drug concentration vs. time. Figure 3 shows the results of the killing curves displayed in figure 2 after mathematical transformation. The additional data points in figure 3 were derived from killing curves (data not shown) obtained in the kinetic model at constant (1 and 2 μg/ml) and decreasing (from initially 1 and 2 μg/ml) concentrations of gentamicin. In summary, a good negative correlation for both modes of dosing was found (for pooled data, $r = 0.954$) between the log of the number of surviving organisms and the log of the AUC. A difference between the two methods of dosing, however, could not be demonstrated with *P. aeruginosa* strain no. ATCC 9721 (table 1). In additional experiments, inocula of 10^6 cfu of *P. aeruginosa*/ml were similarly exposed to decreasing and constant concentrations of gentamicin. These results are also shown in table 1. In some experiments, dilution of the test cultures to be compared was started at 0, 0.5, or 1 hr after addition of the drug to test further the hypothesis that the shape of the drug concentration curve (in the range as seen in vivo) might affect the bactericidal activity of gentamicin. In none of these experiments or in those performed with clinical isolate no. 14974 was a significant difference between constant and decreasing concentrations of gentamicin demonstrated when the effect of similar AUCs was compared. In contrast, a comparison of the regression lines obtained from experiments using large and small inocula of *P. aeruginosa* strain no. ATCC 9721 disclosed a significant difference regarding the bactericidal threshold AUC (minimally active AUC) and slope (table 1). The results of experiments with *P. aeruginosa* no. 14974 were similarly affected by the size of the inoculum.

PAE. In most experiments simulating intermittent dosing, the drug concentration decreased to less than the MAC during the course of the experiment; however, an immediate regrowth was not observed (figure 2). In contrast, the organisms stayed in a lag phase of growth which lasted 1-2 hr. Additional experiments under static conditions were performed to explain this persistent drug effect. *P. aeruginosa* organisms were exposed to gentamicin for a limited interval, after which the drug was removed. Analyses of the regrowth pattern revealed a persistent PAE which lasted for 1.4 hr when lag-phase organisms and 1.6 hr when log-phase organisms had been exposed to a gentamicin concentration of five times the MIC for 30 min (figure 4). When an inoculum of 10^7 cfu/ml was

Table 1. Effect of mode of exposure to drug and inoculum size on the activity of gentamicin against *Pseudomonas aeruginosa* strain no. ATCC 9721.

	Slope		Minimal active AUC*	
Mode of exposure	$10^{7.5}$ cfu/ml	10^6 cfu/ml	$10^{7.5}$ cfu/ml	10^6 cfu/ml
Decreasing	−4.84†	−3.44†	0.77	0.21
Constant	−5.24‡	−3.9‡	0.76	0.21
Pooled	−5.15§	−3.84§	0.77	0.21

NOTE. The bacteria were exposed in vitro to constant or to continuously decreasing (simulating in vivo kinetics) concentrations of gentamicin in the model shown in figure 1. Results from 16 killing curves were plotted as the log of the percentage of surviving organisms vs. the log of the area-under-the-drug-concentration-vs.-time curve (AUC). Resulting regression lines were compared for the significance of differences after analysis of covariance [15] in the *F*-test. At each inoculum size the difference between the two modes of exposure was not significant.

* Bactericidal threshold AUC of gentamicin concentration vs. time (in μg × hr/ml). The values were calculated by regression analysis.

† $P < 0.05$.

‡ $P < 0.05$.

§ $P < 0.01$.

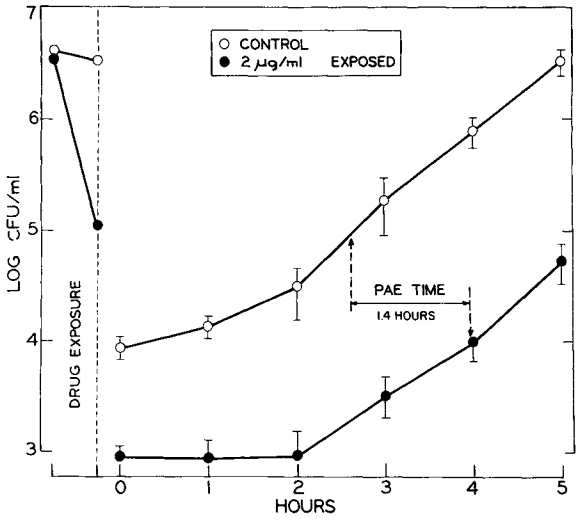

Figure 4. Persistent postantibiotic effect (PAE) of short exposure (30 min) of lag-phase *Pseudomonas aeruginosa* strain no. ATCC 9721 organisms to five times the MIC of gentamicin. Data are means ± SD of triplicate determinations. Arrows indicate the time required for 1-log regrowth to occur in the treated and the control cultures.

exposed for 2 hr to 0.4 μg of gentamicin/ml (corresponding to the MIC for a conventional inoculum), a PAE time of 1.3 hr was still demonstrated.

Regrowth phase. After 6–8 hr of exposure to gentamicin, the test organisms usually started to regrow. Regrowth occurred whether the culture was exposed to constant or decreasing drug concentrations. The regrowing subpopulations were at least partially resistant to a second simulated bolus injection of gentamicin at 8 hr (figure 2). Even a concentration of gentamicin twice that of the initial concentration added at 12 hr (at which time dilution was always stopped) had little or no effect. The selected gentamicin-resistant subpopulations harbored frequently, but not always, small-colony-forming variants. Pure populations of small-colony variants were selected in some experiments, but when subcultured these variants were unstable in morphology and gentamicin resistance.

Discussion

The present study has demonstrated that different methods of dosing of antimicrobial agents can be compared in vitro. Such comparisons are of particular interest for potentially toxic drugs with a small therapeutic index and for microorganisms posing particular therapeutic problems. Hence, we focused on gentamicin and *P. aeruginosa*.

We were unable to demonstrate in vitro a superiority of constant infusion over simulated intermittent injection of similar doses of gentamicin. Rather, keeping the inoculum the same, the effect of gentamicin correlated with the log of the AUC and was not dependent on the mode of how this AUC was obtained. In the model used, the in vivo kinetics of gentamicin were simulated by dilution of the drug, and, as a consequence, the test organisms were diluted as well. A comparison between the two simulated modes of dosing was nevertheless possible. Cultures exposed to decreasing drug concentrations and those exposed to constant drug concentrations in a dynamic steady state were tested in parallel and identically diluted, all cfu determinations were corrected for the inevitable dilution of the bacteria, the comparison was made between MICs and subinhibitory concentrations, and large inocula were also studied. Finally, the comparison was based on identical AUCs. Similar studies, all focusing mainly on β-lactam antibiotics [10, 11, 14, 16, 17], have ignored at least in part these factors, although they are relevant for the evaluation of the antimicrobial efficacy of fluctuating concentrations of drug.

The demonstrated persistent PAE of gentamicin on *P. aeruginosa* was unexpected. It has not been observed with *Staphylococcus aureus* [18]. However, we were able to demonstrate a PAE of gentamicin on strains of *P. aeruginosa* (including no. ATCC 27853) but not on *Escherichia coli* [13].

The regrowth of a gentamicin-selected subpopulation (frequently small-colony variants) of *P. aeruginosa* was a major problem for the interpretation of kinetic experiments looking at repeated dosing. The regrowing population proved to be at least partially gentamicin-resistant. Therefore, experiments in which multiple doses were given, simulating clinical treatment with gentamicin, could not be performed. Because these resistant variants do not inactivate the drug and because ribosomal resistance to gentamicin has, to our knowledge, not been described, we assume that they are defective in aminoglycoside uptake. Such mutants have been investigated by Bryan and Van Den Elzen [19] and Bryan et al. [20].

We examined the effect of different modes of gentamicin dosing on isolated target organisms in vitro. In vivo, additional factors are involved and

might be of paramount importance. Though this proposal is controversial, the mode of aminoglycoside administration might affect the penetration of the drug into tissues and bronchial secretions [21–23]. Further, the oxygen-dependent mechanism of aminoglycoside uptake in susceptible bacteria might well be of minor importance in infected tissues because oxygen can be scarce at these sites [24]; the lag phase of the bactericidal effect of gentamicin could be considerably longer, and the PAE might be absent in vivo. Thus, additional studies in vivo, both in animal models and in patients, are needed to establish the role of dosing regimens on the efficacy of aminoglycoside antibiotics.

References

1. Rodriguez, V., Bodey, G. P. Epidemiology of pseudomonas infections in patients with malignancies. *In* R. G. Doggett [ed.]. *Pseudomonas aeruginosa:* clinical manifestations of infection and current therapy. Academic Press, New York, 1979, p. 369–375.
2. Singer, C., Kaplan, M. H., Armstrong, D. Bacteremia and fungemia complicating neoplastic disease: a study of 364 cases. Am. J. Med. 62:731–742, 1977.
3. Jackson, G. G., Riff, L. J. Pseudomonas bacteremia: pharmacologic and other bases for failure of treatment with gentamicin. J. Infect. Dis. 124(Suppl.):S185–S191, 1971.
4. Flick, M. R., Cluff, L. E. Pseudomonas bacteremia: review of 108 cases. Am. J. Med. 60:501–508, 1976.
5. Bodey, G. P., Chang, H.-Y., Rodriguez, V., Stewart, D. Feasibility of administering aminoglycoside antibiotics by continuous intravenous infusion. Antimicrob. Agents Chemother. 8:328–333, 1975.
6. Feld, R., Tuffnell, P. G., Curtis, J. E., Messner, H. A., Hasselback, R. Empiric therapy for infections in granulocytopenic cancer patients: continuous infusion of amikacin plus cephalothin. Arch. Intern. Med. 139:310–314, 1979.
7. Keating, M. J., Bodey, G. P., Valdivieso, M., Rodriguez, V. A randomized comparative trial of three aminoglycosides – comparison of continuous infusions of gentamicin, amikacin and sisomicin combined with carbenicillin in the treatment of infections in neutropenic patients with malignancies. Medicine (Baltimore) 58:159–170, 1979.
8. Stratton, C. W., Reller, L. B. Serum dilution test for bactericidal activity. I. Selection of a physiologic diluent. J. Infect. Dis. 136:187–195, 1977.
9. Washington, J. A., II, Barry, A. L. Dilution test procedures. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant [ed.]. Manual of clinical microbiology. 2nd ed. American Society for Microbiology, Washington, D.C., 1974, p. 410–417.
10. Grasso, S., Meinardi, G., de Carneri, I., Tamassia, V. New in vitro model to study the effect of antibiotic concentration and rate of elimination on antibacterial activity. Antimicrob. Agents Chemother. 13:570–576, 1978.
11. Bergan, T., Carlsen, I. B., Fuglesang, J. E. An in vitro model for monitoring bacterial responses to antibiotic agents under simulated in vivo conditions. Infection 8(Suppl. 1):S96–S102, 1980.
12. Stevens, P., Young, L. S. Simple method for elimination of aminoglycosides from serum to permit bioassay of other antimicrobial agents. Antimicrob. Agents Chemother. 12:286–287, 1977.
13. Bundtzen, R. W., Gerber, A. U., Cohn, D. L., Craig, W. A. Postantibiotic suppression of bacterial growth. Rev. Infect. Dis. 3:28–37, 1981.
14. Murakawa, T., Sakamoto, H., Hirose, T., Nishida, M. New in vitro kinetic model for evaluating bactericidal efficacy of antibiotics. Antimicrob. Agents Chemother. 18:377–381, 1980.
15. Snedecor, G. W., Cochran, W. G. Statistical methods. 6th ed. Iowa State University Press, Ames, 1967, p. 432–438.
16. Nishida, M., Murakawa, T., Kamimura, T., Okada, N. Bactericidal activity of cephalosporins in an in vitro model simulating serum levels. Antimicrob. Agents Chemother. 14:6–12, 1978.
17. Rifkin, G. D., Pack, G. Pharmacokinetic and bactericidal effects of cephalosporins on a susceptible strain of *Klebsiella pneumoniae*. *In* J. D. Nelson and C. Grassi [ed.]. Current chemotherapy and infectious disease. Vol. 1. American Society for Microbiology, Washington, D.C., 1980, p. 685–686.
18. McDonald, P. J., Craig, W. A., Kunin, C. M. Persistent effect of antibiotics on *Staphylococcus aureus* after exposure for limited periods of time. J. Infect. Dis. 135: 217–223, 1977.
19. Bryan, L. E., Van Den Elzen, H. M. Effects of membrane-energy mutations and cations on streptomycin and gentamicin accumulation by bacteria: a model for entry of streptomycin and gentamicin in susceptible and resistant bacteria. Antimicrob. Agents Chemother. 12:163–177, 1977.
20. Bryan, L. E., Nicas, T., Holloway, B. W., Crowther, C. Aminoglycoside-resistant mutation of *Pseudomonas aeruginosa* defective in cytochrome c_{552} and nitrate reductase. Antimicrob. Agents Chemother. 17:71–79, 1980.
21. Thys, J. P., Mouawad, E., Klatersky, J. Concentrations of netilmicin in bronchial secretions and serum during intermittent vs. continuous infusion: a crossover study in humans. J. Infect. Dis. 140:634, 1979.
22. Pennington, J. E., Reynolds, H. Y. Pharmacokinetics of gentamicin sulfate in bronchial secretions. J. Infect. Dis. 131:158–162, 1975.
23. Bergeron, M. G., Nguyen, B. M., Gauvreau, L. Influence of constant infusion versus bolus injections of antibiotics on in vivo synergy. Infection 6(Suppl. 1):S38–S45, 1978.
24. Hays, R. C., Mandell, G. L. pO_2, pH and redox potential of experimental abscesses. Proc. Soc. Exp. Biol. Med. 147:29–30, 1974.