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Killing of *Pseudomonas aeruginosa* during Continuous and Intermittent Infusion of Ceftazidime in an In Vitro Pharmacokinetic Model

JOHAN W. MOUTON* AND JAN G. DEN HOLLANDER

Department of Clinical Microbiology and Antimicrobial Therapy, Erasmus University Hospital Rotterdam, Rotterdam, The Netherlands

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An in vitro pharmacokinetic model mimicking human serum drug concentrations, based on a dialyzer unit, was developed to study the efficacies of continuous infusion and intermittent administration of ceftazidime over a period of 36 h. The daily dose of ceftazidime was 300 mg/liter/24 h given either as a continuous infusion or as three bolus doses. The intermittent dosing regimen yielded peak and trough concentrations after the fourth dose of 92.3 (standard deviation, 8.0) and 1.4 (standard deviation, 0.9) mg/liter, respectively. Continuous administration yielded concentrations of approximately 20 mg/liter. To study efficacy, three *Pseudomonas aeruginosa* strains, ATCC 27853, CF4, and CF16, were used. The MICs of ceftazidime for these strains were 1, 4, and 16 mg/liter, respectively. Strain CF16 was killed initially during both regimens and then started to regrow. At the end of the fourth dosing interval, i.e., after 32 h, viable counts showed no difference between the regimens. Strains ATCC 27853 and CF4 were killed initially during both dosing schedules, and after the first dosing interval viable counts were similar. However, after the fourth interval, there was a marked difference between bacterial counts during continuous and intermittent infusion, being 2.2 and 2.8 $\log_{10}$, respectively, demonstrating a greater efficacy during continuous infusion. The results indicate that, in the absence of other factors, a sustained level of ceftazidime around or slightly above the MIC is not high enough to maintain efficacy over more than one (8-h) dosing interval. When sustained concentrations higher than four times the MIC are employed, continuous administration in this model is more efficacious than intermittent dosing.

Time-kill curves for beta-lactam antibiotics against *Pseudomonas aeruginosa* show time-dependent killing which is maximal at relatively low concentrations (35). Concentrations much higher than the MIC contribute no extra effect. From these experiments, it can be deduced that continuous serum drug concentrations above the MIC of the antibiotic used to combat the microorganism in question should be more efficacious than declining concentrations, as observed after intermittent dosing (8, 10). During the latter regimen, concentrations fall below the MIC during part of the dosing interval. It has been shown in several animal models that continuous infusion is indeed more efficacious than intermittent dosing (14, 21, 27). However, the half-life of most drugs is severely shortened in the animals studied, and conclusions regarding the pharmacodynamics in humans are difficult to make (15). For, if the shortened half-life is taken into account, the time during which the concentration is below the MIC applies for almost the entire dosing interval, which is contrary to the situation as observed in humans. This problem has, however, been partly overcome by fractional dosing to mimic human pharmacokinetics (13).

In vitro simulation of human pharmacokinetics may thus give additional information with respect to kinetics of killing of microorganisms. Several in vitro pharmacokinetic models to simulate human pharmacokinetics have been described, based on either dilution (3, 4, 16, 17, 20, 23, 26, 28, 32) or diffusion (1, 5, 12, 34, 39). We developed an in vitro pharmacodynamic model based on a dialyzer unit, which is described here. This model was subsequently used to study the efficacy of continuous versus intermittent infusion of ceftazidime against *P. aeruginosa* over a period of 36 h. Although there are some reports suggesting a better efficacy in vitro when beta-lactam antibiotics are dosed continuously (16), in those studies they were given in combination with an aminoglycoside. In other reports, the MIC is compared with the results obtained in the pharmacodynamic model (19, 31, 37), but the circumstances under which the MIC is determined differ from those in the model. Furthermore, all these studies were based on dilution models, and although correction for dilution of the bacteria (36) can be made, artifacts due to dilution have to be precluded (18). In the present study, we used a standard dose yielding concentrations comparable to those observed in humans during treatment. Three *P. aeruginosa* strains requiring different MICs were examined.

MATERIALS AND METHODS

Model. A two-compartment model consisting of one central compartment and several peripheral compartments was designed to expose bacteria to changing antibiotic concentrations mimicking human pharmacokinetics. Figure 1 shows a schematic diagram of the model. Antibiotic was added to a central compartment containing broth. This central compartment was diluted from a diluent reservoir with a peristaltic pump (Econo pump; Biorad, Veenendaal, The Netherlands). Antibiotic-containing broth was pumped from the central compartment to the elimination reservoir at the same pump rate, thus keeping the central compartment at a constant volume. The peripheral compartments consisted of disposable dialyzer units (ST23; Baxter, Utrecht, The Netherlands) with a pore diameter of 2.8 nm. Up to four serially placed dialyzers were used to allow

* Corresponding author. Mailing address: Dept. of Clinical Microbiology and Antimicrobial Therapy, University Hospital Rotterdam, Dijkzigt, Dr. Molenwaterplein 40, 3015 GD Rotterdam, The Netherlands. Phone: 31-(0)10-4633511. Fax: 31-(0)10-4633875.

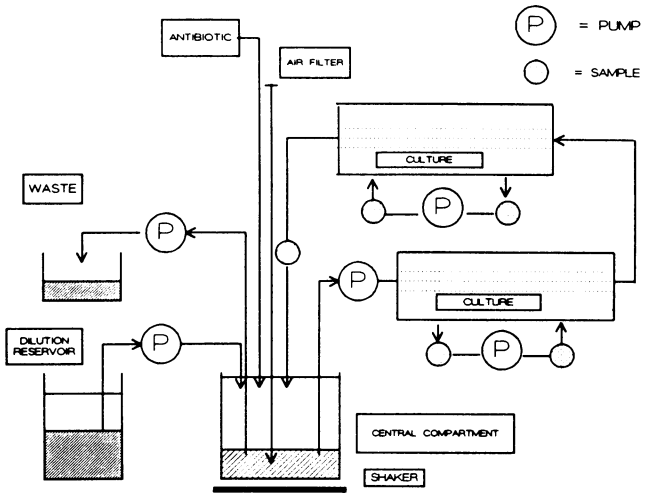

FIG. 1. Schematic diagram of the two-compartment model. Antibiotic is added to the central compartment by bolus injection or by use of a pump. The volume of the central compartment is kept constant by setting the pumps from the dilution reservoir and to the waste reservoir at the same rate, yielding an exponential decline of the concentration of the antibiotic. The central and the peripheral compartments communicate through artificial capillaries. Two peripheral compartments are shown here; in experiments, up to four were placed in series.

simultaneous cultures to be studied. A peristaltic pump (Bio-rad Econo pump; 17.5 ml/min) was used to circulate the culture in each unit. Inoculation and sampling were done through silicone injection points (BSM-V01 S; Hospal, Uden, The Netherlands) with sterile needles. Each peripheral unit contained 150 ml of bacterial culture. The contents of the central compartment were pumped through the artificial capillaries of the dialyzer unit by a peristaltic pump (Rhône Poulenc Medical 440; 125 ml/min). A high flowrate was chosen to obtain a fast equilibrium between the central and peripheral compartments. The central and peripheral compartments were placed on a shaking apparatus (125 rpm) to provide optimum dilution of the injected antibiotic and to accelerate bacterial growth in the peripheral compartments. Air was blown into the broth of the central compartment through an air filter. The complete system was placed in a room at 37°C ambient temperature.

Media. Mueller-Hinton broth (Difco, Amsterdam, The Netherlands) supplemented with Ca^{2+} (22 mg/liter) and Mg^{2+} (12 mg/liter) was used in all experiments (MHB_s).

Strains. *P. aeruginosa* AT1 (ATCC 27853), CF4, and CF16 were used. Strain CF4 was originally isolated from a cystic fibrosis patient. CF16 is a laboratory mutant of strain CF4. The MICs of ceftazidime were 1, 4, and 16 mg/liter for *P. aeruginosa* AT1, CF4, and CF16, respectively. Both CF strains were nonmucoid and showed the same growth characteristics as strain AT1 (see below).

Antibiotic. Ceftazidime was obtained from Glaxo (Zeist, The Netherlands). Stock solutions were prepared according to the guidelines of the National Committee for Clinical Laboratory Standards (24).

MICs and growth curves. MICs were determined by both a standard agar dilution method and a microdilution method (24). Growth curves were made as follows. One colony from a fresh overnight culture on Iso-Sensitest agar (Oxoid CM 471) was suspended in MHB_s and incubated overnight on a shaking apparatus (200 rpm) at 37°C. The inoculum was obtained by diluting this culture to approximately 5×10^5 CFU/ml and incubating at 37°C while shaking. Samples of this log-phase culture were taken at time (t) = 0, 1, 2, 4, 6, and 24 h and diluted serially 10-fold in cold sterile saline on ice. A 100-μl volume of each dilution was plated on Iso-Sensitest agar and incubated overnight at 37°C. Growth curves were also determined in the model described above, only without injection of antibiotic. At t = 0, each of the peripheral compartments was filled with a log-phase culture of approximately 5×10^5 CFU/ml. Samples were taken at t = 0, 1, 2, 4, 6, and 24 h for determination of CFU per ml as described above.

Pharmacokinetic and killing curves. The in vitro activities of changing ceftazidime concentrations against three strains were tested in the model described above. For the intermittent infusion experiment, at t = 0 (immediately after the inoculation of the log-phase cultures into the peripheral compartments) ceftazidime solution (100 mg/liter per dose) was injected in the central compartment. This was repeated after each eighth hour, continuing for 36 h. Samples were taken at t = 0, before and 10 and 30 min after each dose, further at every hour until t = 10 h, then every 2 h until t = 16 h, and at t = 25, 28, 33, and 36 h. For the continuous infusion experiment, at t = 0 an infuser (Braun, The Netherlands) was used, providing the same daily dose of ceftazidime as during intermittent infusion (300 mg/liter/24 h). Every 12 h, the ceftazidime solution in the infuser was replaced. Samples were taken at t = 0, 10 min, 30 min, 1 h, and every hour until t = 10 h, then every 2 h until t = 14 h, and at t = 24, 26, 28, 30, 32, and 36 h. The samples used for viable counts (0.5 ml) were immediately washed (twice) with sterile saline on ice before the 10-fold dilutions were made. The theoretical limit of detection was 4 CFU/ml. At 40 CFU/ml the coefficient of variation was <20%, and at 100 CFU/ml it was <10%. Samples taken for ceftazidime assay were stored at −70°C. Samples were assayed for ceftazidime by high-performance liquid chromatography as described earlier (22). The limit of detection was 0.5 mg/liter, and the coefficient of variation and correlation coefficient in the range 0 to 100 mg/liter were <10% and 0.999, respectively.

Other assays. β-Lactamase production in the original samples was determined qualitatively at t = 0, 8, 14, 24, 32, and 36 h by a standard iodometric method (25). Briefly, a 100-μl sample was added to 100 μl of freshly prepared penicillin G solution (6,000 mg/liter). Two drops of starch solution (1%) were added, and the mixture was incubated at room temperature for 30 to 60 minutes. After addition of iodine reagent, disappearance of the then-blue color of the mixture was considered positive.

The MICs for the isolated strains at these time points and the pH of the medium were also determined.

Pharmacokinetic and statistical analysis. The growth rate (μ) was determined during log phase (7). Pharmacokinetic analysis of the time-concentration curves was done by using the equations of Allen et al. (2). Pharmacokinetic parameters were estimated by using the SAS NLIN computer program package (29) with a one-compartment open model. The area under the concentration-time curve over the first 32 h was determined by using the log-linear trapezoidal rule (2). The t test was used to determine statistical significance between groups. Significance was accepted at P = 0.05 (two-tailed test).

RESULTS

Pharmacokinetic curves. Typical examples of the pharmacokinetic curves obtained during intermittent and continuous infusion are shown in Fig. 2 and 3. The curves were reproduc-

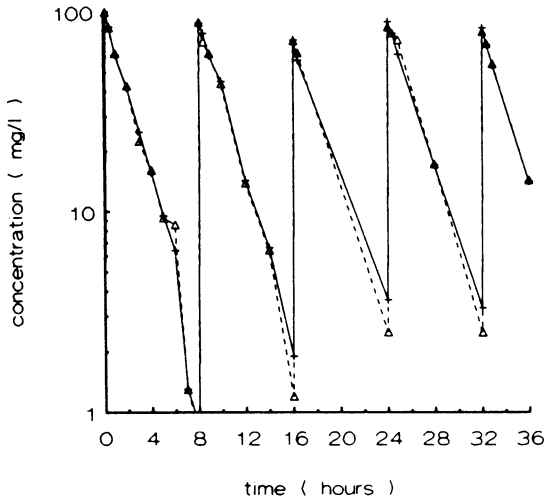

FIG. 2. Typical example of concentration-time curves of ceftazidime during intermittent bolus injection at every eighth hour (300 mg/liter/24 h). Crosses, central compartment; triangles, peripheral compartment.

ible, and the pharmacokinetic parameters in each of the dialyzer units were comparable (Table 1). The peak and trough concentrations during intermittent infusion as well as the half-lives did not differ significantly for the five doses (Table 1). The area under the concentration-time curve from 0 to 32 h was slightly higher during intermittent infusion (678.8 mg · h/liter) (standard deviation [SD], 117.8) than that for continuous infusion (609.5 mg · h/liter [SD, 13.4]). The mean concentration of ceftazidime was 19.8 mg/liter (SD, 1.6) at the end of the fourth dosing interval (Table 1).

Growth curves. The growth rates (±SD, means of four curves) of strains AT1, CF4, and CF16 were 1.07 (SD, 0.27), 0.83 (SD, 0.28), and 1.01 (SD, 0.22) liters/h, respectively, and not statistically significantly different from each other. The growth rates in the model were 1.18 (SD, 0.24), 1.03 (SD, 0.18), and 1.02 (SD, 0.24) liters/h, respectively.

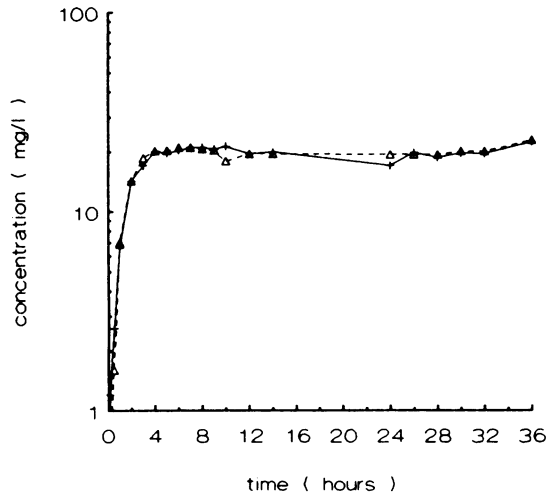

FIG. 3. Typical example of concentration-time curves of ceftazidime during continuous infusion (300 mg/liter/24 h). Crosses, central compartment; triangles, peripheral compartment.

TABLE 1. Pharmacokinetic parameters during intermittent and continuous infusion[a]

Dose	Trough concn (mg/liter)	Peak concn (mg/liter)	$t_{1/2}$ (h)[b]	Mean concn at end of each interval[c] (mg/liter)
1	0	96.3 ± 7.8	1.50 ± 0.23	21.1 ± 2.0
2	0.62 ± 0.16	102.3 ± 14.8	1.49 ± 0.23	19.8 ± 1.1
3	2.2 ± 1.6	84.5 ± 9.8	—[d]	19.7 ± 2.3
4	1.4 ± 0.9	92.3 ± 8.0	1.67 ± 0.32	19.8 ± 1.6
5	2.2 ± 0.8	86.9 ± 7.1	1.56 ± 0.17	21.2 ± 1.9

[a] All values are means ± SD of seven curves.
[b] $t_{1/2}$, half-life.
[c] Continuous infusion.
[d] —, not determined.

Killing curves. Killing curves of the *P. aeruginosa* strains exposed to ceftazidime are shown in Fig. 4 and 5. During intermittent infusion, bacteria were killed initially but started to regrow after 7 h. After each dose, there was some killing or suspension of growth, but then growth was resumed; this was most apparent for strain CF16. During continuous infusion, strains AT1 and CF4, for which the MICs were 1 and 4 mg/liter, respectively, were killed initially and then started to regrow for a few hours, whereupon the number of CFU per milliliter remained constant during the remainder of the experiment. After the first dosing interval of 8 h, there was no difference in killing by continuous and intermittent infusion for strains AT1 and CF4. In contrast, after each subsequent dosing interval, there was an increasing difference between the efficacies of intermittent and continuous infusion in favor of the latter, which was most marked after the last dosing interval, i.e., after 32 h (Table 2). The difference was somewhat less for strain AT1 than for strain CF4 because of the emergence of a resistant mutant in one experiment whereby the MIC of ceftazidime increased to 64 mg/liter (see below). Strain CF16

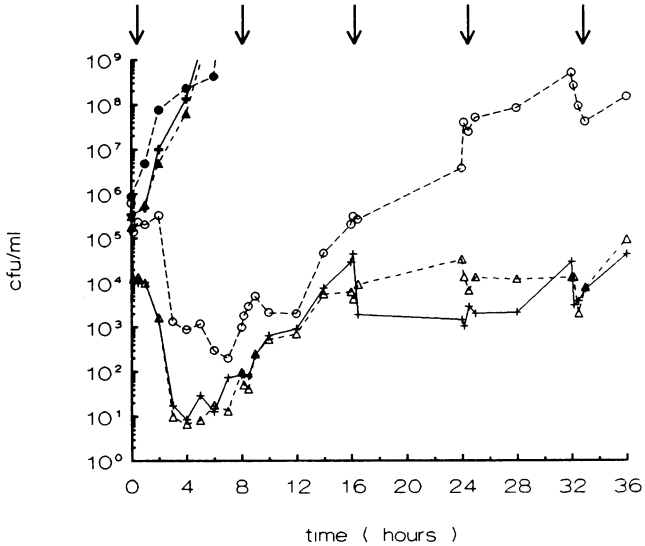

FIG. 4. Killing curves for *P. aeruginosa* AT1 CF4, and CF16 (thin crosses, open triangles, and open circles, respectively) and growth controls (boldface crosses and corresponding closed symbols) during intermittent bolus injection. Data shown are geometric means of at least two experiments.

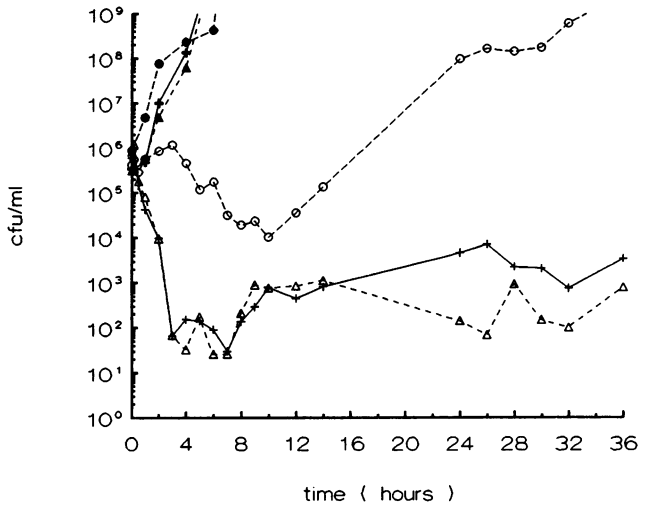

FIG. 5. Killing curves for *P. aeruginosa* AT1, CF4, and CF16 and growth controls during continuous infusion. Data shown are geometric means of at least two experiments. Symbols are as described for Fig. 4.

was killed initially, but during continuous infusion numbers of bacteria were not reduced to the same extent as during intermittent infusion. Regrowth started at approximately the same time during both regimens, and after 32 h the numbers of CFU per milliliter were equal (Table 2).

Emergence of resistant strains. To determine whether resistant strains emerged in the course of time, the MICs for strains isolated during the experiments were determined. The MIC for the AT1 strain remained constant, except in one experiment during continuous infusion. The MICs for the strains isolated during the experiments with CF4 and CF16 increased during both continuous and intermittent infusion and amounted to 128 mg/liter after 24 h. Thereafter no strains requiring higher MICs could be isolated. Determination of β-lactamase in the medium of CF16 strain revealed that a detectable amount of β-lactamase was present at the time of isolation of the resistant strains but not before. β-Lactamase could also be detected after the emergence of the resistant AT1 strain during continuous infusion. In contrast, β-lactamase could not be detected in the samples from the experiments with strain CF4, during either continuous or intermittent infusion. pH measurements of the samples showed no deviating values.

TABLE 2. Changes in bacterial concentrations during treatment with intermittent and continuous dosing regimens of ceftazidime[a]

Strain (MIC, mg/liter)	Interval (h)	Change in bacterial concn[b]		
		Intermittent	Continuous	Difference
ATCC 27853 (1)	8	3.3	3.7	0.4
	32	0.7	2.9	2.2[c]
CF4 (4)	8	3.3	3.6	0.3
	32	1.1	3.9	2.8[d]
CF16 (16)	8	2.8	1.3	−1.5
	32	−2.9	−3.2	−0.3

[a] Changes between 0 and 8 h (after one dosing interval) and 0 and 32 h (after 4 dosing intervals).
[b] Given as change in $\log_{10}$ CFU per milliliter between $t = 0$ h and $t = 8$ h or $t = 32$ h (geometric mean of at least two experiments).
[c] $P < 0.05$ for the strains not producing β-lactamase.
[d] $P < 0.05$.

DISCUSSION

Several models have been used to mimic drug concentrations in plasma in humans (1, 3–5, 12, 16, 17, 20, 23, 26, 28, 32, 34, 39). The model developed here, based on diffusion, enables the study of killing kinetics during continuous and intermittent dosing regimens. The model proved to adequately reproduce pharmacokinetic profiles.

In the present study, the model was used to determine whether continuous administration of ceftazidime is more efficacious than intermittent dosing, using dosing regimens yielding concentrations comparable to those observed in vivo. The half-life chosen for these experiments was approximately 1.5 h. This is in accordance with values found in vivo (11, 22, 33). The daily dose chosen yielded concentrations comparable to those found in vivo. The killing curves of *P. aeruginosa* show that, during the time course of the experiment, continuous infusion was more efficacious than intermittent infusion for strains AT1 and CF4. Although a bolus dose initially killed bacteria, the *P. aeruginosa* strains started to regrow after several hours. At each new dose, growth was halted for some time or some killing was achieved, but then the bacteria resumed growing. A similar observation was made for amoxicillin (19, 31) and ampicillin (34, 37) against *Escherichia coli*. After the start of continuous infusion, bacteria were killed as well, but regrowth was substantially less or hardly present during continuous infusion compared with that during intermittent infusion. This is in agreement with the hypothesis and some observations that gram-negative organisms exposed to beta-lactams resume growth when concentrations fall below a certain level (9) and that high concentrations do not exert an additional effect as, for instance, for the quinolones (6).

The most consistent index of bactericidal activity found by White et al. (37), when studying efficacy of ampicillin against *E. coli*, was the area under the concentration-time curve and not the time above the MIC (T_{MIC}). The T_{MIC} did not covary strongly with the MIC, and the authors suggest that the high frequency of emergence of resistant strains may have masked the importance of the T_{MIC}. Since during continuous infusion the T_{MIC} was incessant for strains AT1 and CF4 at the start of the experiments, and continuous infusion was more efficacious than intermittent infusion, we conclude that the T_{MIC} is an important parameter. The suggestion of White et al. (37) that their failure to find the T_{MIC} to be an important parameter may have been caused by the high frequency of emergence of resistant strains may therefore be correct. Studying the efficacy of beta-lactams in patients with pneumonia, Schentag et al. (30) also found the T_{MIC} to be important.

It has been argued that the emergence of resistance, or the continued isolation of strains, is possibly an artifact of in vitro systems due to the adherence of bacteria to the walls of the incubation flasks (18). This observation was made in dilution models, and control experiments in this type of model can rule this out (18, 37). In the diffusion model used in the present study, the importance of adherence of bacteria is not easily assessable. However, if adherence were a main determinant of the outcome of the studies, one might expect that there would have been no difference in the outcome of the dosage regimens.

To determine whether the MIC itself may influence the interaction between bacteria and antibiotic, we examined three strains. *P. aeruginosa* CF4 and CF16 were 4- and 16-fold less susceptible to ceftazidime, respectively, than strain AT1, and the growth rates were comparable with each other. Although the concentration during continuous infusion was maintained at approximately 20 mg of ceftazidime per liter, which is higher

than the MIC for strain CF16, this particular strain started to regrow after some time. This was due to the appearance of strains requiring MICs up to 128 mg/liter, a phenomenon that was also observed during intermittent infusion. Regrowth due to resistant subpopulations has also been observed in other studies with a comparable model. However, in those studies the bacteria were exposed to enoxacin and gentamicin, which belong to another group of antibiotics (6, 38). β-Lactamase, not detectable in the earlier samples, was present in the medium from approximately the same time onwards, possibly facilitating regrowth. These results also indicate that the apparent resistance was probably due to the production of β-lactamase by *P. aeruginosa*. This was probably due to the emergence of a derepressed mutant, as was determined by exposure after cefoxitin (results not shown). The regrowth during continuous administration, despite continuous concentrations above the MIC, implies that these concentrations are not high enough to prevent regrowth.

In contrast to this observation for strain CF16 was the appearance of resistant strains of CF4 requiring a high MIC during both continuous and intermittent infusion. These strains were highly resistant, as determined by the MIC, yet appreciable growth did not occur. The absence of growth of the isolated mutants of CF4 is not easy to explain. It could be that if the experiment had lasted longer regrowth would have been observed, although this did not occur during the 12 h that the strain was present. The presence of β-lactamase could not be detected, suggesting that resistance was cell wall mediated in these cases.

The results of these experiments suggest that when the antibiotic is the only agent present to exert efficacy, as is the case in neutropenic patients, a sustained level around or slightly above the MIC is not high enough. The strains exposed to a sustained concentration of five times the MIC or more were inhibited in their growth, indicating that a concentration four to five times the MIC is effective. When sustained concentrations higher than five times the MIC are employed, continuous administration in this model is more efficacious than intermittent dosing.

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