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Alteration of Postantibiotic Effect during One Dosing Interval of Tobramycin, Simulated in an In Vitro Pharmacokinetic Model

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The kinetics of the postantibiotic effect (PAE) during one dosing interval of tobramycin against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was investigated. We determined the PAE at different time points during this dosing interval of 12 h in an in vitro pharmacokinetic model simulating human pharmacokinetics in which the half-life of tobramycin was adjusted to 2.4 ± 0.2 h. Using an enzymatic method to inactivate tobramycin, we determined PAEs in samples extracted from the model at 1, 5, 8, and 12 h, corresponding with tobramycin concentrations of 20, 5, 2, and 1 times the MIC for the test organism. The PAE decreased significantly from 2.5 h at 1 h to 0 h at 12 h. No change in MIC was observed for the strains during the experiments. We conclude that the PAE decreases with decreasing tobramycin concentrations during a 12-h dosing interval and completely disappears after the concentration has reached the MIC for the test organism. On the basis of these observations, the emphasis that is placed on the PAE in discussions about the optimal dosing interval in aminoglycoside therapy is questionable.

Three factors are relevant when determining an aminoglycoside dosing schedule, i.e., the susceptibility of the bacterium (MIC), the kinetics of antibacterial activity, and the postantibiotic effect (PAE) (2). The PAE is usually determined after 1 or 2 h of exposure to antibiotics at four times the MIC (4×MIC) to 5×MIC followed by immediate removal or inactivation of the antibiotic. Partly on the basis of these data, intermittent therapy of aminoglycosides with longer dosing intervals, such as once- or twice-daily dosing, has been introduced into clinical practice (7, 8, 16, 19) since the PAE supposedly would prevent bacterial growth when drug concentrations in serum and tissue fall below the MIC (1). However, the PAE is determined in vitro after 1 or 2 h of exposure to fixed antibiotic concentrations while in the clinical setting aminoglycoside concentrations decline over time, with an elimination half-life of several hours. It could well be that the PAE has disappeared by the time the concentration falls below the MIC, thus losing its clinical relevance.

In the present study, the PAE was determined during one dosing interval of tobramycin in a pharmacokinetic model simulating human pharmacokinetics. The PAE was determined at four time points during this dosing interval of 12 h to study the kinetics of the PAE itself and to determine whether the PAE is still present when the drug concentration falls below the MIC for the infecting organism.

The strains used for this experiment were *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. The MIC of tobramycin (Eli Lilly and Company, Nieuwegein, The Netherlands) was 0.5 mg/liter for *S. aureus* and 0.5 mg/liter for *P. aeruginosa*, determined by both a standard macrodilution method with Mueller-Hinton broth (MHB) supplemented with Ca^{2+} (25 mg/liter) and Mg^{2+} (12.5 mg/liter) (Difco, Amsterdam, The Netherlands) and an agar dilution method (15).

The model used was described previously in detail (14). Briefly, a two-compartment model consisting of one central compartment and four peripheral compartments consisting of disposable dialyzer units (ST23; Baxter, Utrecht, The Netherlands) was used to expose the bacteria in the peripheral compartments to changing antibiotic concentrations, mimicking human pharmacokinetics. A volume of 150 ml of a logarithmic-phase culture of *S. aureus* or *P. aeruginosa* at a concentration of approximately 10^7 CFU/ml was injected into the peripheral compartments starting at 0 h, tobramycin was infused into the central compartment for 1 h with an infuser (Braun, Melsungen AG, Germany), providing a total dose of 15 mg/liter and resulting in a peak concentration of approximately 10 mg/liter at 1 h. The half-life of tobramycin was adjusted to approximately 2 h. To determine the tobramycin concentration by a fluorescence polarization immunoassay using a TDxFLx device (Abbott Diagnostic Division, Amstelveen, The Netherlands) and colony counts, samples were taken at 0, 10, and 30 min and 1, 5, 8, and 12 h from both the central and peripheral compartments. To determine the killing rates for the two strains, the samples were washed two times with cold saline, diluted serially 10-fold in cold saline, and plated on tryptone soy agar plates (Oxoid, Basingstoke, Hampshire, England) for colony count determination. Control growth curves were also determined in the pharmacokinetic model.

To determine the PAE, samples were taken at 1, 5, 8, and 12 h, corresponding with estimated tobramycin concentrations of 20×MIC (peak), 5×MIC, 2×MIC, and 1×MIC (trough), respectively. An enzymatic method for the removal of tobramycin was used (5). Briefly, each 1-ml sample was added to 9 ml of Mueller-Hinton broth at 37°C. To this medium a tobramycin-acetylating enzyme [AAC(3)-II] (12) and acetyl coenzyme A were added, resulting in the inactivation of tobramycin within 5 min. The PAE was defined according to Craig and Gudmundsson (3) as follows: PAE = $T - C$, where T is the

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TABLE 1. Tobramycin PAEs for *S. aureus* and *P. aeruginosa*

Time (h)	Tobramycin concn[a] (mg/liter)	Tobramycin concn/MIC	PAE[b] (h) for:	
			S. aureus	*P. aeruginosa*
1	10.6 (1.1)	21.2	2.6 (1.8–3.3)	2.5 (1.9–3.1)
5	2.6 (0.4)	5.2	0.7 (0.2–1.2)[c]	0.9 (0.6–1.2)[c]
8	1.1 (0.2)	2.2	0.6 (0.1–1.0)[c]	1.5 (1.1–1.9)[c]
12	0.5 (0.1)	1.0	0.1 (−0.1–0.3)[c]	−0.3 (−0.6–0.0)[c]

[a] Data are means of four separate experiments performed in duplicate. Values in parentheses are standard deviations.
[b] Data are means of four separate experiments performed in duplicate. Values in parentheses are 95% confidence intervals.
[c] Significant decrease compared with the PAE at 1 h.

time required for the CFU count in the test culture to increase 1 $\log_{10}$ above the count immediately after drug removal and *C* is the corresponding time for the controls. From the PAE regrowth cultures, samples were taken at 0, 1, 2, 3, 4, and 6 h and diluted serially in cold saline. Each dilution was plated on tryptone soy agar and incubated overnight at 37°C.

Control growth curves were determined in the pharmacokinetic model, which was treated as the test cultures were, only without the addition of tobramycin. For the determination of PAE control curves, samples were taken at 0, 1, 5, 8, and 12 h and diluted in Mueller-Hinton broth at 37°C until the inoculum had the same starting value for CFU per milliliter as the test strain did at the time, thus ruling out the possibility of an inoculum effect. As a control, 1 ml of this inoculum was treated as the test strains were by adding tobramycin-acetylating enzyme and acetyl coenzyme A. The experiment was repeated four times in duplicate for each strain. MICs were determined by a standard agar dilution method (15) for the strains isolated from the samples at 1, 5, 8, and 12 h.

The SAS computer package (18) was used for statistical analysis. The PAEs at four different time points were analyzed by the Tukey-Kramer multiple-comparison test and the values determined for regrowth during the killing curve were analyzed by repeated measurement and analysis of variance.

The mean concentrations ± standard deviations for tobramycin in the four experiments in the pharmacokinetic model are shown in Table 1. The peak and trough concentrations as well as the half-lives did not vary significantly between separate experiments. The actual measured half-life ± the standard deviation was 2.4 ± 0.2 h, which is slightly longer than the adjusted half-life but is still within the range of half-lives found for patients.

Killing and regrowth curves of *S. aureus* and *P. aeruginosa* were determined at 1, 5, 8, and 12 h. A representative example of a killing and a regrowth curve of both strains in the pharmacokinetic model is shown in Fig. 1. The figure also shows an example of a control growth curve and its regrowth curve, which were determined separately. The regrowth which is seen in the killing curve in the model is significant over time from 1 to 12 h ($P = 0.0022$) for *P. aeruginosa* but not for *S. aureus* during all experiments.

A summary of all PAEs determined at all time points is given in Table 1. For both *S. aureus* and *P. aeruginosa*, the PAE after a 1-hour exposure at a tobramycin concentration of 20×MIC was approximately 2.5 h. The PAEs determined at 12 h were essentially 0 h. Thus, the PAE of tobramycin decreases rapidly over time in concordance with the concentration-versus-MIC ratio in this pharmacokinetic model. The MICs of tobramycin for *S. aureus* and *P. aeruginosa* isolated from the samples at 1, 5, 8, and 12 h were 0.5 mg/liter. No genotypically resistant strains were isolated during the experiments.

In this study, we describe the result that the PAE decreases to 0 h during one dosing interval of tobramycin. PAEs determined at the peak concentration of about 10.6 mg/liter were approximately 2.5 h for both *S. aureus* and *P. aeruginosa*, slightly higher than those found in literature when determined at 4×MIC to 5×MIC (3). The PAE at the peak concentration is determined after an exposure of 1 h, but at a final concentration of 20×MIC. This could explain the slight difference relative to the PAEs determined at 4×MIC to 5×MIC. During the dosing interval, however, PAEs decreased to 0 h at 12 h, i.e., when the antibiotic concentration is approximately the MIC. Apparently, during a half-life of 2.4 h the bacteria are able to recover more rapidly than the time it takes for the concentration of tobramycin to decrease to the MIC for these strains.

The PAEs determined during in vivo experiments in animal models are produced in rodents with a short antibiotic half-life (significantly shorter than that for humans) (3). But if the half-life for the rodents is adjusted to that for humans by

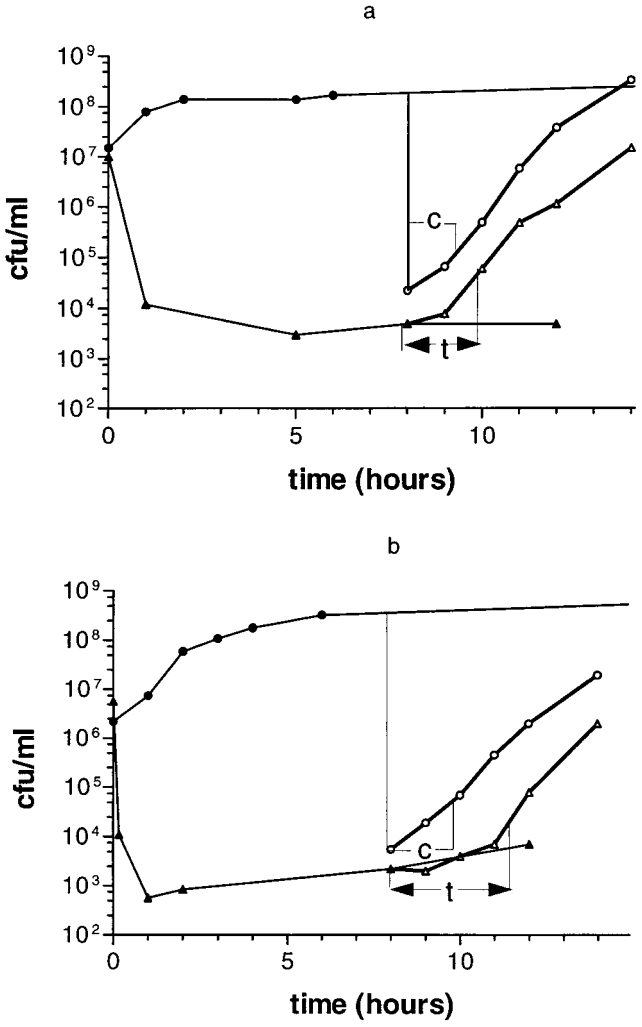

FIG. 1. Representative curves for a control growth (●), control regrowth (○), killing (▲), and killing regrowth (△) of *S. aureus* (a) and *P. aeruginosa* (b) as determined during all experiments in the pharmacokinetic model are shown. For these curves, samples for PAE measurement were taken at 8 h. PAE can be determined as PAE = $T - C$, according to Craig and Gudmundsson (3) (see text for details).

inducing renal impairment, the PAEs become even longer (4), so the half-life cannot be used as an explanation for the declining of the PAE in the in vitro model.

The PAE not only declines during a dosing interval but also disappears after multiple antibiotic exposures (9, 11, 13, 17). However, in previous studies (9, 11, 13, 17) the bacterial cultures were repeatedly exposed to the same concentration of antibiotic for short periods of time (2 h).

The killing curve of *P. aeruginosa* as determined in the model (Fig. 1) shows a significant log-linear trend ($P < 0.0001$) from 5 to 12 h. At the beginning of the dosing interval, bacteria are killed rapidly, and this is followed by a lag phase induced by tobramycin (from 1 to 5 h) during which the number of CFU per milliliter does not change significantly in the model. During the ensuing period bacterial growth accelerates, leading to a significant increase in CFU per milliliter from 5 to 12 h. This could be due to regrowth of phenotypically resistant subpopulations, since growth was observed at tobramycin concentrations exceeding the MIC. After subculturing, we did not observe an emergence in genotypical resistance to tobramycin in the organisms. The occurrence of phenotypically resistant subpopulations cannot be excluded, as plating the samples on tobramycin-containing agar was not performed. Thus, we cannot confirm the observation of Karlowsky et al. (10) of a MIC reversion time. The fact that regrowth of *P. aeruginosa* occurs at tobramycin concentrations exceeding the MIC supports the argument that keeping the tobramycin concentration above the MIC is not the most important parameter of efficacy. The fast initial killing caused by relatively high concentrations of aminoglycosides is possibly one of the most important factors during therapy with these drugs. Another reason for this regrowth phenomenon could be the difference in inoculum sizes used for the MIC determination and for the PAE determination, 5×10^5 and 1×10^7 CFU/ml, respectively, since the use of a larger inoculum could result in a higher MIC (the true MIC). The MIC determined with an inoculum of 10^7 CFU/ml was 1.0 mg/liter for both strains (data not shown). This however explains only the regrowth of *P. aeruginosa* observed after 8 h, when the tobramycin concentration decreases to <1 mg/liter.

In order to optimize the use of aminoglycoside antibiotics, results of several studies investigating their pharmacodynamic parameters predicting efficacy (1, 9, 10) and toxicological effect (6) are being used. Dosing schedules have been determined by matching the pharmacokinetic profile of a drug in normal volunteers to the drug's activity in vitro (20). The decrease of the PAE to 0 h during a complete dosing interval might deflate the importance which is given to the PAE in discussions about the dosing interval in aminoglycoside therapy. The results of this study do not argue against a change of thrice-daily to once-daily dosing schedules, but they do question the role of the PAE in its rationale. Thus, once-daily therapy can still be favored on the basis of reduced toxicity (6) and higher peak levels, producing a higher initial killing rate that may well be clinically highly relevant (10).

In conclusion, the present study describes a significant decrease in PAE from 2.5 to 0 h during one dosing interval of tobramycin. The important impact which is ascribed to the PAE in discussions about the lengthening of the dosing interval is therefore questioned.

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