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Betty J. McGrath

Randall Marchbanks
University of Rhode Island

Deborah H. Gilbert
University of Rhode Island

Michael N. Dudley
University of Rhode Island

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In Vitro Postantibiotic Effect Following Repeated Exposure to Imipenem, Temafloxacin, and Tobramycin

BETTY J. McGRATH,† C. RANDALL MARCHBANKS, DEBORAH GILBERT, AND MICHAEL N. DUDLEY*

Antiinfective Pharmacology Research Unit, University of Rhode Island College of Pharmacy, Roger Williams Medical Center, Providence, Rhode Island 02908

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The postantibiotic effect (PAE) following three consecutive 2-h exposures to imipenem, temafloxacin, and tobramycin was determined in *Pseudomonas aeruginosa*. A PAE and a bactericidal effect were consistently observed for imipenem following each cycle of drug exposure and regrowth. In contrast, the PAE increased with repeated exposure with temafloxacin (1.8 to >5 h), but disappeared with tobramycin by the third exposure (0.9 to 0 h). These data show that the in vitro PAE may change within a strain following multiple cycles of drug exposure and bacterial regrowth.

The design of optimal dosage regimens for antibiotics used in the management of infectious diseases is dependent upon determination of pharmacokinetic and pharmacodynamic parameters which reproducibly predict efficacy while minimizing risks for the development of toxicity. Bacterial killing rates and the postantibiotic effect (PAE) are examples of important pharmacodynamic parameters which aid in the optimization of dosing regimens (4).

While the PAE has often been determined following a single "dose" or exposure, the effect of repeated antibiotic exposures to bacteria on the PAE has not been evaluated. Since most infections are treated with multiple doses of antibiotics which result in several cycles of drug exposure (and in some cases regrowth), it is important to consider the reproducibility of the PAE in the design of dosage regimens. The aim of the present study was to determine how the PAE for *Pseudomonas aeruginosa* is affected following repeated antibiotic exposure with three agents representative of classes of drugs exerting a PAE: imipenem, temafloxacin, and tobramycin.

A clinical isolate of *P. aeruginosa* (strain 92) isolated from a patient at Roger Williams General Hospital and the laboratory strain *P. aeruginosa* ATCC 27853 were studied. Imipenem (lot 80595) and temafloxacin (lot A-63004) were supplied by Merck Sharp & Dohme (West Point, Pa.) and Abbott Laboratories (Abbott Park, Ill.), respectively. Tobramycin (lot OD29810) was obtained from Sigma Chemical Co. (St. Louis, Mo.). Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with calcium (50 mg/liter) and magnesium (24 mg/liter) (MHB-S) was used for all susceptibility and PAE studies. The CFU per milliliter was determined by using unsupplemented Mueller-Hinton agar (MHA).

Pre-exposure MICs were determined by the broth macrodilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (6) by using a starting inoculum of $\sim 5 \times 10^5$ CFU/ml (6). The MIC was defined as the lowest concentration of antibiotic that prevented visible growth following an 18-h incubation at 37°C.

Bacterial inocula were prepared from a 20-h overnight growth of three to five colonies picked from an agar plate, diluted 1:1,000 in MHB-S, and incubated for 3 h at 37°C to bring the organisms into a log-linear growth phase. Five to six replicates of antimicrobial agent-exposed cultures were performed for each drug. The starting inoculum for each drug-bacterium combination was adjusted to give a 1×10^6 to 1×10^7 CFU/ml in a total volume of 10 ml of MHB-S. The concentrations of antibiotics were chosen such that a PAE of 1 to 2 h would be produced after the first cycle of drug exposure. Pilot experiments determined that exposure to 10× the MIC (20 μg/ml) of imipenem, 4× the MIC (8 μg/ml) of temafloxacin, and 2× the MIC (2 μg/ml) of tobramycin for 2 h at 37°C resulted in PAEs within this range for the test strains. Bacteria were incubated in tubes with or without drugs (control) for 2 h.

Following a 2-h incubation of drug plus bacteria, the tubes were centrifuged at 1,200 × *g* for 15 min, and 9 ml of supernatant was removed. The bacteria contained in the remaining 1-ml aliquot were resuspended in 9 ml of fresh, prewarmed, drug-free MHB-S and were gently vortexed. This procedure was repeated three times. To ensure comparability of growth patterns of the control and the drug-exposed cultures, the control-growth tube was diluted in drug-free MHB-S after the washing procedure to bring the bacterial concentration in the control-growth tube to the same level as that in the drug-exposed tube.

The effects of a second and a third cycle of drug exposure and removal on the PAE were assessed when drug-exposed cultures had regrown to the level of the initial inoculum prior to the first cycle of drug exposure. One milliliter of a concentrated stock solution of drug was added to the culture tube containing regrowing bacteria at 6 to 8 h (second exposure) or 14 to 16 h (third exposure). The mixture was vortexed and incubated for 2 h. After the 2-h incubation, drug-containing and control cultures were processed and samples were collected as described above following the first exposure.

The number of bacteria was quantified by removing 100 μl from the culture at hourly intervals for the first 4 h and after the drug was removed. Samples were serially diluted with cold 0.9% sodium chloride, and 20 μl was plated onto MHA in triplicate. The plates were incubated for 18 to 24 h, and the colonies were counted. When bacterial counts were ex-

* Corresponding author.
† Present address: Department of Pharmacy 1B, Detroit Receiving Hospital, Detroit, MI 48201.

pected to be low, 100-μl samples were placed in 10 ml of cold 0.9% sodium chloride and were drawn through a 0.45-μm-pore-size filter (Millipore, Danvers, Mass.) under suction. Filters were placed aseptically on MHA and incubated for 18 to 24 h, and the colonies were counted. The lower limit of detection of the number of bacteria for this procedure was 10 CFU/ml. The PAE was calculated from plots of CFU per milliliter versus time by classical methods (1).

The susceptibilities of the isolates recovered during the regrowth phase following the first through third exposure cycles were determined by the broth macrodilution method. The isolates were prepared for MIC testing by growing them overnight in MHB-S, and the isolates were tested as described above.

Imipenem produced a 2- to 3-log-unit decrease in the initial CFU per milliliter with each exposure. The PAEs were between 1 and 3 h for both strains and did not change with repeated cycles of drug exposure and removal (Fig. 1). There were no significant changes in the susceptibility of either strain to the drug.

In contrast to imipenem, there were significant changes in the bacterial killing and PAEs for both temafloxacin and tobramycin with repeated drug exposure-growth cycles. With tobramycin, bacterial killing with the third exposure was reduced; a 4-log-unit decrease in the CFU per milliliter was observed following the first exposure to tobramycin, with no bactericidal activity observed following the third exposure (Fig. 2A). While the initial exposure to tobramycin produced a PAE of ca. 1 h, the PAE was lost by the third exposure; this loss of a PAE was associated with an increase in the MIC from 1 to 8 mg/liter.

A third pattern of change was observed with the fluoroquinolone temafloxacin. Although the initial exposure produced marked bacterial killing, a less than 1-log-unit CFU-per-milliliter reduction was observed during the third cycle of exposure (Fig. 2B). The bacteriostasis induced following the third exposure resulted in a prolonged PAE that was estimated to be over 5 h (Fig. 2B). The pre- and postexposure MICs of temafloxacin differed by only twofold.

The pharmacodynamics of antimicrobial agents have become recognized as important elements for consideration in the design of optimal drug dosage regimens. In particular, the PAE is one pharmacodynamic parameter which has led to the exploration of dosage regimens that use dosing intervals that are extended beyond that predicted from consideration of drug levels in serum and indices of drug effect, such as the MIC. One assumption, implicit in the clinical application of the PAE in using more prolonged dosage intervals, is that the value of the PAE would be reproducible and unchanged throughout a course of treatment.

The results presented in this report demonstrate that the in vitro PAE may change following several cycles of drug exposure and bacterial regrowth. With temafloxacin and tobramycin, but not imipenem, the changes in the PAE occurred as soon as the second or third cycle of drug exposure. These classes of agents may select for drug-resistant subpopulations of *P. aeruginosa* in vitro and in vivo (3). Although the simultaneous disappearance of a PAE and loss of a bactericidal effect has also been noted previously for imipenem (7), we did not observe this in our study.

In contrast, the loss of a PAE with tobramycin against *P. aeruginosa* was associated with a reduced bactericidal effect and susceptibility to drug. The changes in bacterial killing were not consistent with the adaptive resistance that occurs following initial exposure to aminoglycosides, as described by Daikos et al. (2). Adaptive resistance occurs during the

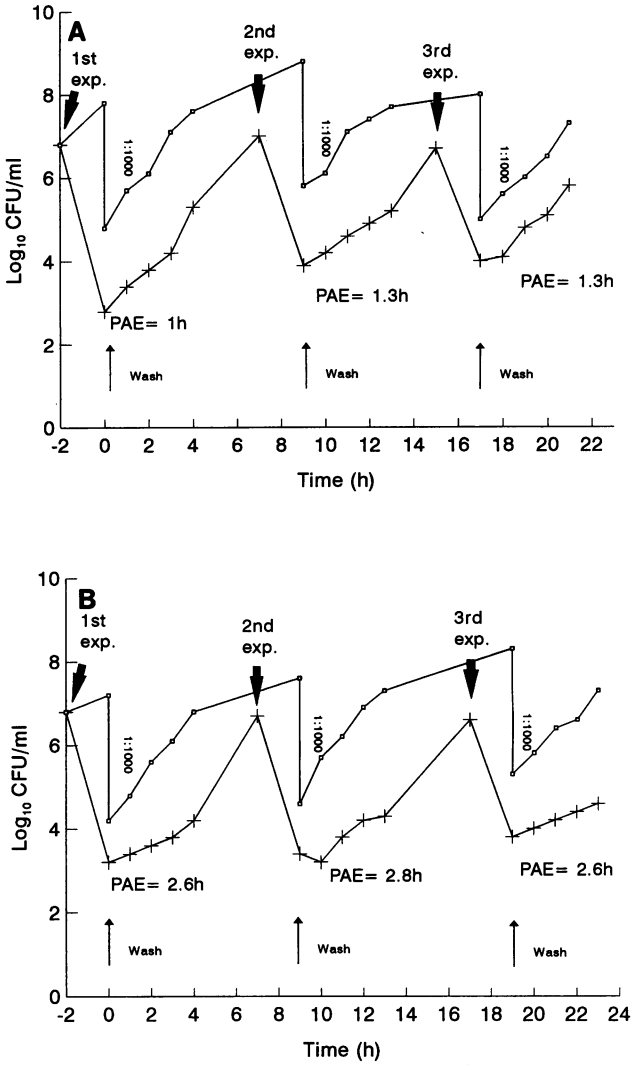

FIG. 1. PAE following repeated cycles of imipenem exposure (exp.) and regrowth for two strains of *P. aeruginosa*, strain 92 (A) and ATCC 27853 (B). Drug-exposed cultures (+) were washed and resuspended in drug-free medium after three 2-h exposures. Control growth cultures (□) were also washed, resuspended in drug-free medium, and diluted (1:1,000) to provide CFU per milliliter similar to that in drug-exposed cultures at the indicated times. Each datum point represents the geometric mean CFU per milliliter of five to six replicates performed over 2 or 3 different days.

early phases of aminoglycoside exposure from down-regulation of drug uptake. Adaptive resistance is present immediately following the PAE, when bacterial regrowth has begun, and ultimately disappears over several hours (2). In contrast to the information presented above, the loss of a PAE and the bactericidal effect with tobramycin in the present study occurred after the period of adaptive resistance would have been expected to have occurred. Bacteria recovered at the end of the experiment had an eightfold reduction in their susceptibilities to tobramycin, suggesting that selection of stable drug-resistant subpopulations had occurred.

Variability and changes in the PAE between and within strains have been demonstrated. It has been shown for gram-negative bacteria that certain aminoglycosides may variably produce a PAE (3, 5). Odenholt-Tornqvist et al. (8)

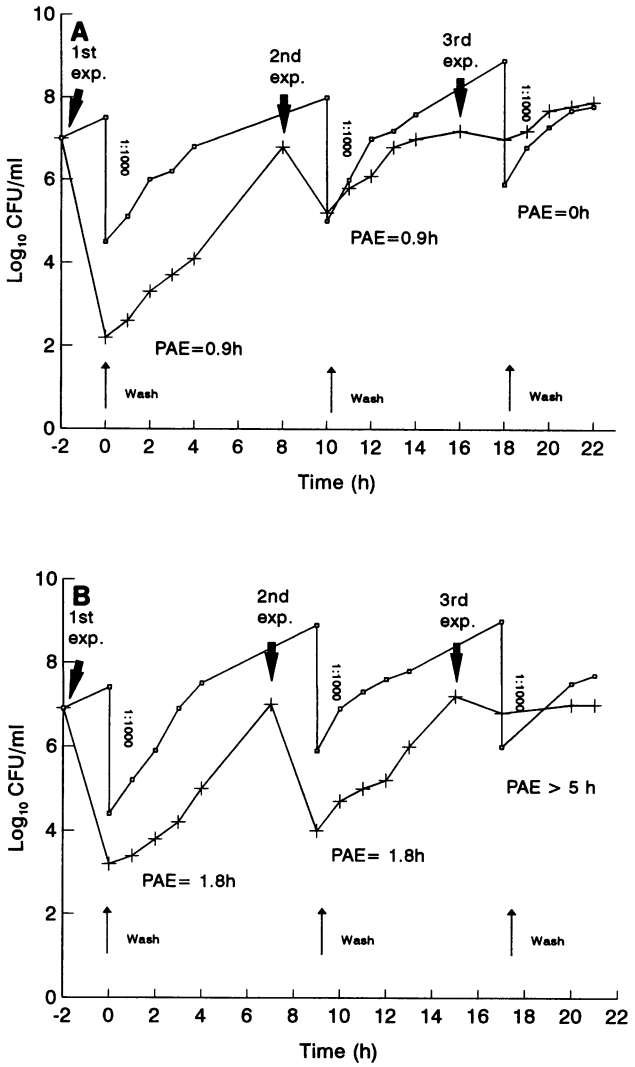

FIG. 2. PAE following repeated cycles of drug exposure and regrowth of *P. aeruginosa* 92 for tobramycin (at 2× the MIC [A]) and temafloxacin (4× the MIC [B]). See the legend to Fig. 1 for descriptions of symbols and procedures.

have also shown changes in the pharmacodynamic effects of beta-lactam antibiotics with exposure of bacteria to supra-MICs of drug that are followed by periods of subinhibitory concentrations. In contrast to these results, Vogelman et al. (9) have confirmed the presence of an in vivo PAE after two doses of gentamicin against *Escherichia coli* and *Klebsiella* species in an experimental thigh infection model.

These data show the limitations of values for the in vitro PAE when derived from a single cycle of drug exposure and regrowth. While several studies in animal and in vitro models of infection and in humans have demonstrated the efficacy of single daily dosing with aminoglycosides (for which a prolonged PAE can be shown), the actual link between demonstration of an in vitro PAE and the efficacies of more prolonged dosage intervals has not been proven. The specific value of the PAE may not be useful precisely for calculating the actual dosage interval for drug administration. Rather, the first-exposure PAE may serve as a marker which should prompt exploration of novel dosage regimens in established in vitro and animal models of infection and, ultimately, in humans.

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