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The Postantibiotic Effect of Imipenem: Relationship with Drug Concentration, Duration of Exposure, and MIC

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The postantibiotic effect (PAE) of imipenem against *Escherichia coli* was measured at a wide variety of drug concentrations and times of exposure. We observed that the area under the concentration-time curve of drug exposure (AUC), the product of time of exposure and concentration of drug, is a much better predictor of the duration of the PAE than either parameter alone. We also measured the PAE of imipenem against strains of gram-positive and gram-negative bacteria for which MICs varied widely. The E_{50}, the AUC required to produce 50% of the maximum PAE, is correlated with the MIC and is independent of species. This may explain why the duration of the PAE differs for bacteria of the same species for which MICs are different.

The postantibiotic effect (PAE), defined as the period of bacterial growth suppression after brief exposure to antimicrobials, has been studied extensively since it was rediscovered in the mid-1970s (8). It occurs with virtually all antibacterial agents against susceptible bacteria, except for penicillins and cephalosporins against gram-negative organisms and streptococci (4). Some studies have demonstrated apparent dose-response relationships between the duration of the PAE and the drug concentration, time of exposure, and/or their product, the area under the concentration-time curve (AUC) of drug exposure (2, 5, 6, 9). However, none of these studies has attempted to define these relationships precisely or determine which parameters best define them.

For β-lactams, studies with animal models have shown that

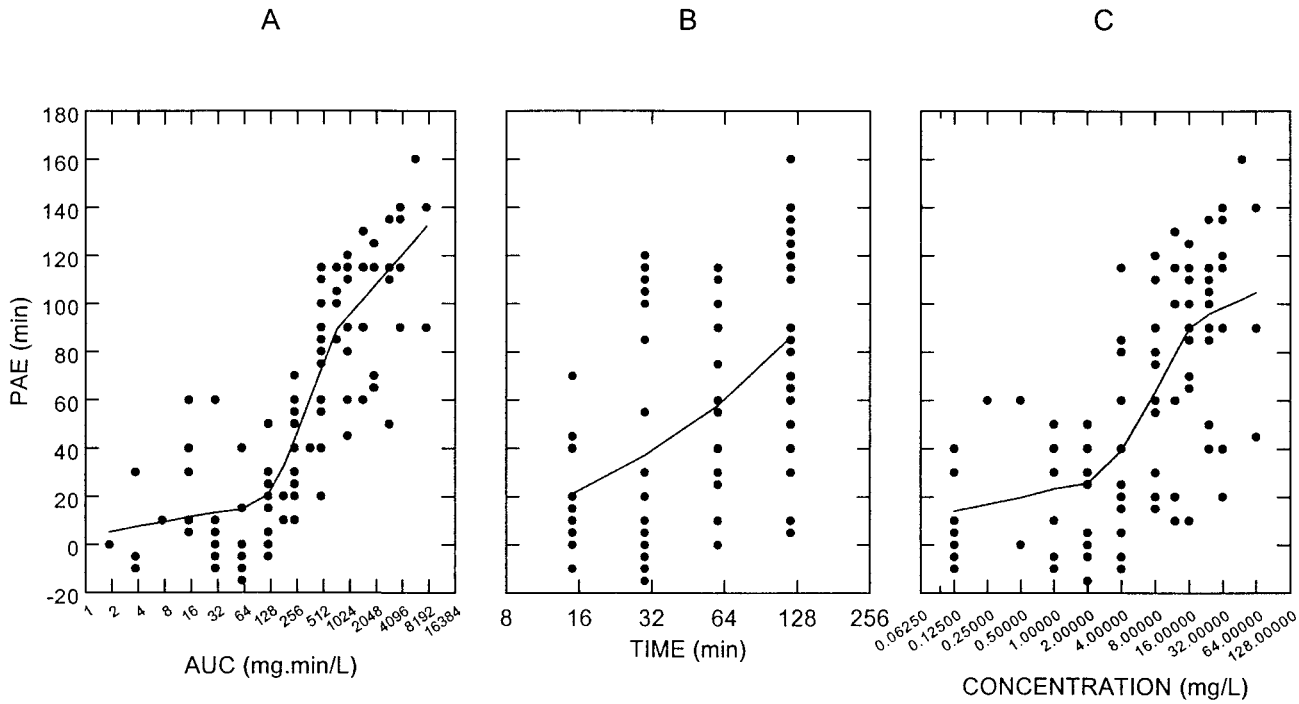

FIG. 1. Relationships between the PAE of imipenem against *E. coli* ATCC 25922 and the AUC (A), time of exposure (B), and imipenem concentration (C).

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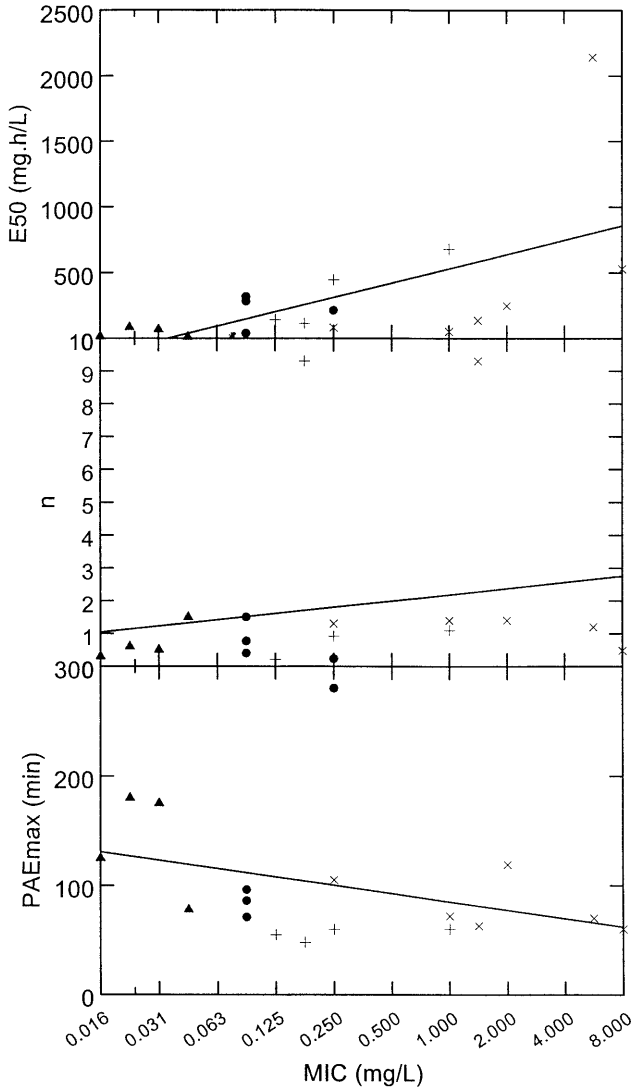

FIG. 2. Relationships between imipenem MIC and E_{50} (AUC required to achieve 50% of the maximum PAE), n (a constant utilized in the Hill equation), and the maximum PAE. ▲, *S. aureus*, +, *K. pneumoniae*; ×, *P. aeruginosa*; ●, *E. coli.*

the major determinant of killing in vivo is time of exposure, expressed as the time that the concentration exceeds the MIC, and not peak drug concentration or AUC (7, 13). By contrast, one early study suggested that the PAE of penicillin against *Staphylococcus aureus* was related to both time and concentration (2). In order to clarify which drug exposure variable correlated best with duration of PAE, we tested imipenem against wild and type strains of *Escherichia coli* at a wide range of drug concentrations and times of exposure. In addition, we further examined the relationships between PAE exposure-response parameters and the MICs for several gram-positive and gram-negative bacteria for which MICs varied widely in order to test whether PAE is related in any way to MIC. We selected imipenem because carbapenems are unique among β-lactams in their ability to produce PAEs in both gram-positive and gram-negative bacteria and because imipenem was the first β-lactam shown to produce a PAE against any gram-negative bacterium (3). The Hill equation was used throughout our study to model the exposure-response relationship (11).

MATERIALS AND METHODS

Measurement of PAE. The in vitro PAE experiments were performed by standard methods (4). In brief, 10^6 CFU of logarithmic-phase organisms per ml was exposed to imipenem in Mueller-Hinton broth at 37°C. After the selected time of exposure had elapsed, drug was removed by centrifuging the solution for 10 min at 2,000 × *g*, decanting the supernatant, and resuspending the organisms in fresh warm broth. This washing procedure was performed twice. Washing was selected as the preferred method of drug removal to avoid carryover of drug from the very high concentrations of drug used in many experiments. After drug removal, viable counts were performed hourly until visible regrowth had occurred. The following controls were included for each experiment: (i) a growth control, prepared and treated similarly to the test solution but without exposure to antibiotic, and (ii) a residual antibiotic control, to which 1/1,000 of the test antimicrobial concentration was added after centrifugation and washing. This last control was included to ensure that after centrifugation and washing the residual drug in the tubes containing the treated organisms did not affect the rate of growth. The PAE was calculated with the standard formula of Craig and Gudmundsson, namely, by deducting the time that the unexposed control took to increase 1 $\log_{10}$ unit (4).

Exposure variable experiments. Two strains of *E. coli*, ATCC 25922 and a clinical strain, were exposed for 15 min and 2 h to concentrations of imipenem ranging from 1 to 700 times the MIC. Overall, tests of 36 different combinations of time of exposure and drug concentration were performed, all of which were repeated 1 to 3 times.

MIC correlation experiments. The following organisms were examined: four strains of *E. coli* (including ATCC 25922 and ATCC 35218), four strains of *Klebsiella pneumoniae* (including ATCC 13883), four strains of *S. aureus* (including ATCC 29213), and six strains of *Pseudomonas aeruginosa* (including ATCC 27853). Apart from the ATCC strains, all were clinical isolates. MICs were determined by a commercial gradient strip method (Etest; AB Biodisk, Solna, Sweden) (1). Where possible, strains for which the MICs differed were selected for study. Test organisms were exposed to imipenem concentrations ranging from 1 to 700 times the MIC for 1 h. Experiments with each strain were repeated 1 to 3 times.

Exposure-response modelling. The Hill equation was used to model the exposure-response curve:

$$\text{PAE} = \frac{\text{PAE}_{\max} \times (\text{exposure variable})^n}{(E_{50})^n + (\text{exposure variable})^n}$$

where $\text{PAE}_{\max}$ is the maximum PAE, E_{50} is the parameter value at which 50% of the maximum PAE is reached, and n is a constant associated with the steepness of the exposure-response curve. The exposure variables time of exposure and drug concentration and their product (the AUC of drug exposure) were modelled separately. The parameters $\text{PAE}_{\max}$, E_{50}, and n were estimated with the nonlinear regression module of Systat for Windows, version 5.1 (Systat Inc., Evanston, Ill.).

Correlation of PAE with MIC. Based on the results of the exposure variable experiments, AUC was selected as the exposure variable from which the parameters $\text{PAE}_{\max}$, E_{50}, and n were estimated. These parameters were estimated for each of the 18 bacterial strains by nonlinear regression of the PAE versus the AUC of drug exposure with the Hill equation. MICs were then compared to each of the three parameters by linear regression performed in Systat.

RESULTS

The results of the exposure variable experiments with *E. coli* ATCC 25922 are shown graphically in Fig. 1. From the nonlinear regression analysis of PAE, the correlation coefficients (corrected r^2 values) for drug concentration, time of exposure, and AUC were 0.000, 0.278, and 0.741, respectively. When the experiments were repeated with a clinical strain of *E. coli*, the correlation coefficients obtained were similar (0.108, 0.331, and 0.729, respectively). Thus, AUC was the best predictor of PAE and was much better than concentration alone, for which there was no correlation, and time alone, for which there was poor correlation.

Results for the MIC correlation experiments with the different bacterial strains are shown in Fig. 2. Curve fitting utilizing the Hill equation was statistically significant, with $P < 0.001$ for each organism (except for P values of 0.01 for one clinical strain each of *S. aureus* and *K. pneumoniae*). Linear regression of the three Hill parameters against MIC revealed

that there was almost no correlation with n (corrected r^2 = 0.000; P = 0.841) and PAE_{max} (corrected r^2 = 0.005; P = 0.312) but moderately good correlation with E_{50} (corrected r^2 = 0.369; P = 0.004).

DISCUSSION

Previous studies examining *S. aureus* and penicillin, *E. coli* and rifampin (2), and *Streptococcus pneumoniae* and roxithromycin (6) have suggested that there is a dose-response relationship between the AUC of drug exposure and the PAE. Others have produced similar findings with a variety of β-lactams, including imipenem against *E. coli* (5). By examining all possible exposure variables, we have shown that AUC is indeed the best determinant of PAE for imipenem. This is somewhat surprising given that time above the MIC, rather than the AUC of drug exposure, is the major pharmacodynamic predictor of efficacy for β-lactams, including carbapenems (7). It is possible that the small amount of concentration-dependent effects exhibited by β-lactams, up to 3- to 4-fold the MIC before maximum effects are achieved, make a contribution to the PAE (10).

We have also shown that E_{50}, the AUC required to produce 50% of the maximum PAE, is correlated with the MIC and is independent of species. It also explains why the duration of the PAE differs for bacteria of the same species for which MICs are different. This was quite noticeable for the strains of *P. aeruginosa*, which were deliberately selected because the MICs for these strains exhibited a broad range. This correlation of E_{50} with MIC was expected, as it has been shown that the EC_{50} for the bacterial killing rate (the concentration required to produce 50% of maximum killing) correlates with the MIC (12).

An important confirmatory part of our findings is the demonstration that in vitro the PAE is an exposure-response phenomenon and that PAE duration varies for different drug concentrations and durations of exposure. Many studies of in vitro PAE examine few or only one concentration and time of exposure and do not define the limits of the effect, especially the maximum effect. The existence of a maximum effect must have implications for dosage schedule design.

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