ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1984, p. 678–682
0066-4804/84/110678-05$02.00/0

Vol. 26, No. 5

Postantibiotic Effect of Imipenem on *Pseudomonas aeruginosa*

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Received 11 June 1984/Accepted 7 August 1984

Imipenem (formerly *N*-formimidoyl thienamycin) and ceftazidime were investigated for their postantibiotic effect on *Pseudomonas aeruginosa*. Four strains of *P. aeruginosa* in the logarithmic phase of growth were exposed for 1 and 2 h to concentrations of antibiotics achievable in human serum. Recovery periods of test cultures were evaluated after dilution or addition of β-lactamase. A consistent postantibiotic effect against all strains was obtained with imipenem but not with ceftazidime. Although ceftazidime did not have a postantibiotic effect, it did suppress the growth of the organisms at concentrations equivalent to one-third of the MIC. The clinical implications of these effects need further evaluation.

Early studies demonstrated that staphylococci exposed to lethal concentrations of penicillin did not immediately resume growth after the drug was removed (11, 12). Eagle and Musselman (5) later showed that this effect was not peculiar to staphylococci but could also be observed with other species.

More recent studies have characterized the postantibiotic effect (PAE) for gram-positive and gram-negative aerobic organisms (3, 4, 9, 15). Of interest, inhibitors of protein and RNA syntheses have produced the longest PAE for most species (4). β-Lactam antibiotics produce a consistent PAE against gram-positive cocci when used at concentrations near the MIC, but none or a minimal effect was seen against gram-negative bacilli (3). The duration of the PAE of ampicillin for *Escherichia coli* was less than 0.5 h despite exposing these organisms to concentrations 100 times their MIC (15).

Pseudomonas aeruginosa, a significant pathogen for the cancer patient who is granulocytopenic, exhibited a PAE when exposed to gentamicin; but no or a negative PAE was demonstrated when the organism was exposed to ticarcillin, cefoperazone, or moxalactam (4). McDonald and his colleagues have previously demonstrated a short PAE with imipenem against a single strain of *P. aeruginosa* (10).

This study was designed to evaluate two newer β-lactam antibiotics, imipenem (formerly *N*-formimidoyl thienamycin) and ceftazidime, for the presence or absence of a PAE against *P. aeruginosa*. Moxalactam and amikacin also were included as negative and positive controls, respectively.

MATERIALS AND METHODS

Antibiotics. Imipenem was provided by Merck Sharp & Dohme (West Point, Pa.); ceftazidime, by Glaxo Pharmaceuticals (Research Triangle Park, N.C.); amikacin, by Bristol Laboratories (Syracuse, N.Y.); and moxalactam, by Eli Lilly & Co. (Indianapolis, Ind.).

Organisms. Four strains of *P. aeruginosa* were used in all experiments: ATCC 27853 and three clinical isolates from the Veterans Administration Medical Center, Baltimore, Md. (strains 228, 6, and 13). Strain 228 was previously used in an animal model evaluation of imipenem and moxalactam (8). Strains 6 and 13 were randomly selected isolates from the clinical microbiology laboratory of the Baltimore Veterans Administration Medical Center.

Media. Cation (Mg^{2+}, Ca^{2+})-supplemented Mueller-Hinton broth was used for test cultures, controls, and antibiotic stock solutions. Pour plates of Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) were used for colony counts.

MIC determination. MICs were determined by a microtiter broth dilution technique, using a final inoculum of approximately 10^5 to 10^6 bacteria per ml. Briefly, twofold serial dilutions of the antibiotic in 0.05 ml were made with an automated microdiluter. After the addition of 0.05 ml of Mueller-Hinton broth to each well, the inoculum was added with a microdiluter inoculator (Dynatech Laboratories, Inc., Alexandria, Va.). The MIC was the lowest concentration which prevented visible growth after overnight incubation.

Determination of PAE. At time zero, 2 ml of the inoculum was added to 50-ml flasks containing 18 ml of Mueller-Hinton broth with or without test antibiotic. Antibiotic concentrations were 20 μg/ml for imipenem and amikacin, 65 μg/ml for ceftazidime, and 100 μg/ml for moxalactam. These concentrations were chosen to approximate those achieved at 1 h postinfusion in the serum of normal human volunteers who were given intravenous doses of imipenem (1 g), ceftazidime and moxalactam (2 g), and amikacin (7.5 mg/kg). The inoculum was a 2-ml aliquot of a 1:10 dilution of a faintly turbid broth culture in the logarithmic phase of growth, producing a final concentration of inoculum in the test flask of approximately 10^5 organisms per ml. After incubation for 1 h in a 37°C shaking water bath, antibiotics were removed by diluting the contents of the flask by 10^{-2} or 10^{-3} or both with antibiotic-free medium.

The study was repeated for ceftazidime and imipenem. For this evaluation, however, the flasks were incubated with antibiotics for 2 h rather than 1 h and the antibiotics were removed by the addition of 2 ml of broad-spectrum β-lactamase (Whatman Biochemicals, Ltd., Maidstone, Kent, England). To assure that the antibiotics were inactivated after the addition of β-lactamase, imipenem and ceftazidime concentrations were determined by high-pressure liquid chromatography and bioassay. A modification of the technique described by Bennett et al. (2) was used for the bioassay, with *Bacillus subtilis* as the indicator organism for imipenem and *Morganella morganii* as that for ceftazidime.

Bacterial counts of the flask contents were determined at

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TABLE 1. MICs for *P. aeruginosa* strains used in PAE experiments

Antimicrobial agent	MIC (μg/ml) for strain:			
	ATCC 27853	228	6	13
Imipenem	4	2	2	2
Ceftazidime	1	4	2	2
Moxalactam	8	8	8	16
Amikacin	4	8	4	4

time zero, immediately before and after antibiotic removal and each hour after removal for 7 to 8 h, and at 24 h by a pour plate technique, using 10-fold dilutions in phosphate-buffered saline.

Quantitation of the PAE. The PAE was measured, as described by McDonald et al. (9), as the difference in time required by test and control cultures to increase by 1 log after the antibiotic was removed by dilution or after the addition of β-lactamase (9). Bundtzen et al have demonstrated that, for removal of the antibiotic, the addition of β-lactamase or dilution to concentrations far below the MIC are equivalent methods for demonstrating the PAE (4); consequently, for these studies they were considered interchangeable.

The following calculations were used for quantitation of the PAEs of the drugs: (i) T = time (hours) required for the CFU count in the test culture to increase by 1 $\log_{10}$ above the count immediately after dilution or inactivation by β-lactamase; (ii) C = time (hours) required for the CFU count in the control culture to increase by 1 $\log_{10}$ above the count immediately after dilution or addition of β-lactamase; (iii) $T - C$ = time interval during which the drug is seen to affect bacterial growth after exposure. This calculation, defined as the PAE, was used as a basis for comparison of persistent effects of all antibiotics tested. This method prevents interference from the regrowth phenomenon for *P. aeruginosa* exposed to β-lactams noted by Gwynn et al. (6).

Killing curves. Bacterial counts from the undiluted test flask were removed at times as described above, and killing curves were constructed from the resulting colony counts.

RESULTS

Microbiological activity. The MICs for the four strains of *P. aeruginosa* are shown in Table 1. All antibiotic concentrations used to determine the PAE were in excess of the MICs for all strains tested. These concentrations were 5 to 10 times the MIC for imipenem, 2.5 to 5 times the MIC for amikacin, 15 to 65 times the MIC for ceftazidime, and at least 6 times the MIC for moxalactam.

Killing curves. Calculated cumulative log kills against the four strains of *P. aeruginosa* are shown in Table 2. Imipenem and amikacin produced a rapid onset of killing, whereas ceftazidime and moxalactam showed a lag period of approximately 1 h to the onset of bacterial killing.

TABLE 2. Cumulative log kill against four strains of *P. aeruginosa*

Antimicrobial agent	Decrease in CFU/ml (mean ± SD) $\log_{10}$ at:		
	1 h	2 h	7 h
Imipenem	2.17 ± 0.48	2.14 ± 0.64	3.61 ± 1.58
Ceftazidime	0.36 ± 0.28	1.41 ± 0.40	2.95 ± 1.65
Moxalactam	0.46 ± 0.19	1.46 ± 0.30	1.34 ± 0.32
Amikacin	3.64 ± 0.64	4.43 ± 0.50	4.05 ± 1.79

Exposure for 1 h: antibiotic removal by dilution. Imipenem showed a consistent PAE against all strains which varied between 1 h against strain ATCC 27853 and 2.7 h against strain 13 (Table 3). Ceftazidime produced no PAE against any of the strains tested. However, for ceftazidime a prolonged suppression of growth was observed against two of the strains when a 10^{-2} dilution of the culture was used, resulting in a final concentration of approximately one-third of the MIC. This suppressive effect was not seen when a 10^{-3} dilution was used (Fig. 1). Amikacin produced 1- and 3.2-h PAEs against strains ATCC 27853 and 228, respectively. For the other two strains a PAE could not be determined because counts were <10 CFU/ml during the counting procedure.

Moxalactam produced a 0.5-h PAE against strain 13 but not against the remaining strains. These strains showed a more rapid rate of growth when compared with control cultures during the first hour after drug removal (negative PAE) (Fig. 2).

Exposure for 2 h: drug inactivation by β-lactamase. A PAE against all four strains of *P. aeruginosa* was observed after a 2-h exposure to imipenem (Table 4). Figure 3 shows the regrowth curves for strain 228 after exposure to imipenem and ceftazidime. A PAE was consistently present with imipenem and averaged 2.2 h for the four strains. With ceftazidime the PAE was never greater than 0.2 h and averaged −0.1 h (Table 4). Two strains demonstrated a negative PAE similar to that obtained after 1-h exposure. Residual antibiotic did not account for the PAE observed as both imipenem and ceftazidime showed no biological activity or assayable drug by either bioassay or high-pressure liquid chromatography (imipenem) within 5 min after the addition of β-lactamase.

The PAE determination was repeated on a different day for the ATCC strain. The two observations differed by 15% (of the more conservative determination).

DISCUSSION

Our experiments indicate that imipenem possesses a significant PAE against the four strains of *P. aeruginosa* tested. In contrast, moxalactam, as previously described (4), and ceftazidime produced little or no PAE.

The factors which mediate the presence or absence of a PAE among β-lactams are not clear. In contrast to imipenem, moxalactam and ceftazidime were found to produce

TABLE 3. *P. aeruginosa* exposed for 1 h to four antibiotics[a]

Antimicrobial agent	PAE (h) against strain:			
	ATCC 27853[b]	228[c]	6[d]	13[e]
Imipenem	1.0	1.5	2.0	2.7
Ceftazidime	−0.4	−0.1	−0.7	−0.2
Moxalactam	−0.3	−0.2	−1.0	0.5
Amikacin	1.0	3.2	NE[f]	NE

[a] Drugs were removed by dilution.
[b] Final drug concentration after dilution: imipenem, 1/200 MIC; ceftazidime, 1/15 MIC; moxalactam, 1/80 MIC; amikacin, 1/200 MIC.
[c] Final drug concentration after dilution: imipenem, 1/100 MIC; ceftazidime, 1/30 MIC; moxalactam, 1/80 MIC; amikacin, 1/400 MIC.
[d] Final drug concentration after dilution: imipenem, 1/10 MIC; ceftazidime, 1/30 MIC; moxalactam, 1/8 MIC; amikacin, 1/200 MIC.
[e] Final drug concentration after dilution: imipenem, 1/10 MIC; ceftazidime, 1/30 MIC; moxalactam, 1/16 MIC; amikacin, 1/200 MIC.
[f] NE, Not evaluable (counts below 10 CFU/ml).

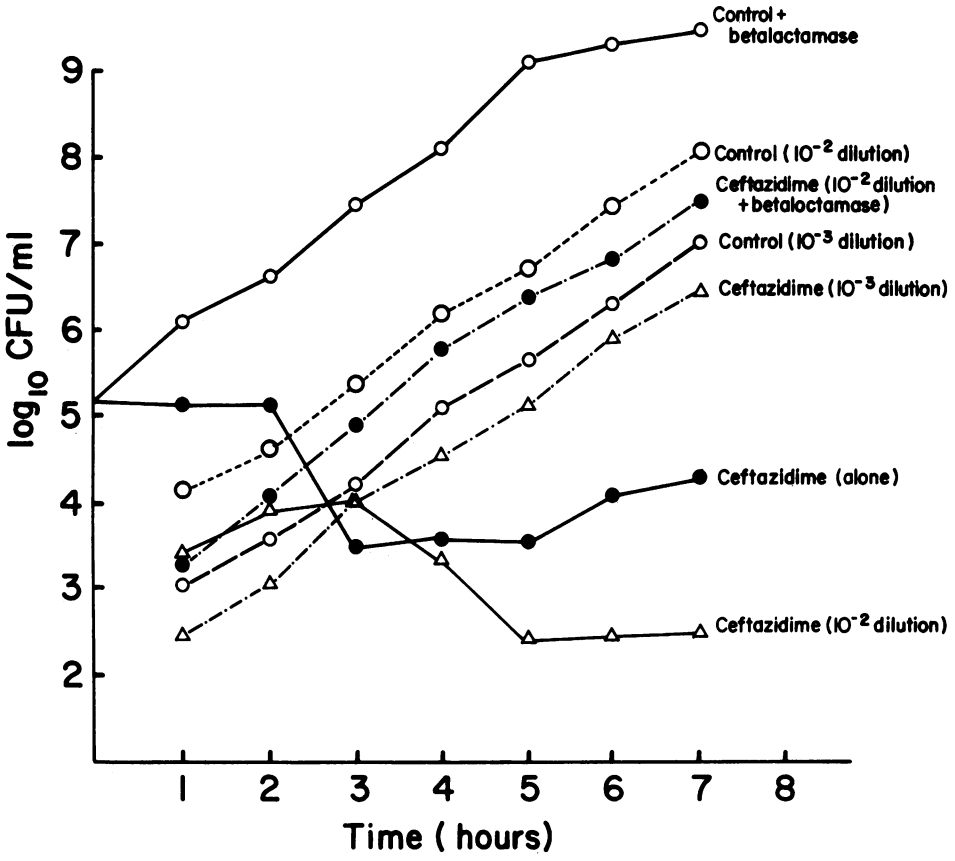

FIG. 1. *P. aeruginosa* strain 6 exposed for 1 h to ceftazidime. Drug removal was effected by dilution plus β-lactamase addition.

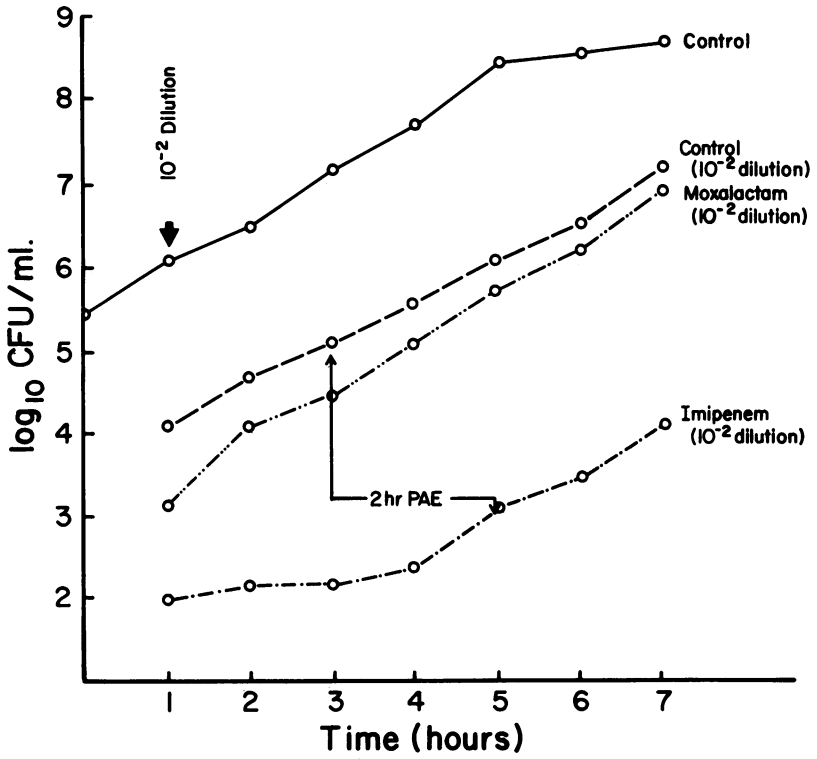

FIG. 2. *P. aeruginosa* strain 6 exposed for 1 h to imipenem and moxalactam. Drug removal was effected by dilution (10^{-2}).

TABLE 4. *P. aeruginosa* exposed for 2 to imipenem and ceftazidime[a]

Antimicrobial agent	PAE (h) against strain:			
	ATCC 27853	228	6	13
Imipenem	2.7	2.2	1.3	2.5
Ceftazidime	0.1	−0.1	−0.7	0.2

[a] Drugs were removed by β-lactamase addition.

no PAE or a negative PAE. Some speculation can be made for this observation. Both moxalactam and ceftazidime bind preferentially to penicillin-binding protein 3, resulting in the formation of long filamentous cells (14); upon removal of these antibiotics, rapid septation probably occurs (up to 12 separate cells per filament) as shown with piperacillin-induced *E. coli* filaments (7). Thus there is a rapid (greater than log phase) increase in CFU, resulting in a "negative" PAE.

Imipenem, like amdinocillin (formerly mecillinam), binds with great affinity to penicillin-binding protein 2, resulting in the formation of enlarged ovoid cells which rapidly lyse by a process that requires cyclic AMP (1). Determination of damage to this particular enzymatic pathway by imipenem and how it may correlate with the presence of a PAE against *P. aeruginosa* requires more investigation.

The clinical significance of these effects has not been established. However, a PAE may be beneficial when dosing intervals are used such that concentrations of the drug at the site of infection become very low; yet, because of the PAE, the regrowth of the organisms would still be prevented.

On the other hand, those β-lactam antibiotics which do not induce a PAE would seem to require the continuous presence of the drug (by means of short dosing intervals or constant infusion) since regrowth occurs immediately after their concentration decreases below one-half to one-eighth of the MIC (13). Examples of this were shown in a recent study by Craig and his colleagues (W. A. Craig et al., Proc. Int. Congr. Chemother. 13th, Vienna, Austria, p. 31/40–31/43, 1983). The PAE was evaluated in a neutropenic mouse thigh infection model. No in vivo PAE could be demonstrated for gram-negative bacilli (including *P. aeruginosa*) when β-lactam antibiotics (i.e., cefoperazone and ticarcillin) were used for treatment. This was in marked contrast to the 2- to 8-h in vivo PAE observed with aminoglycosides.

In attempting to evaluate the importance of the PAE of imipenem in vivo, one may speculate that the presence of a PAE or the absence of a lag period before the onset of rapid kill may have contributed to the results observed by Johnson et al. (8) in a neutropenic rat model of pseudomonas bacteremia. In that study, imipenem resulted in improved outcome in comparison to moxalactam. Drugs were administered at 8-h intervals and serum concentrations were above the MBC for only 2 h. Imipenem provided effective therapy for the lethal *P. aeruginosa* infection, survival being significantly better than for moxalactam-treated animals. These results could not be attributed to higher or more prolonged serum levels of imipenem or to greater in vitro activity against the infecting pathogen.

Imipenem, with both a rapid onset of kill and an ability to produce a significant PAE against *P. aeruginosa*, can be considered to be unique at this time among the newer β-lactams. Deeper understanding of these features may assist

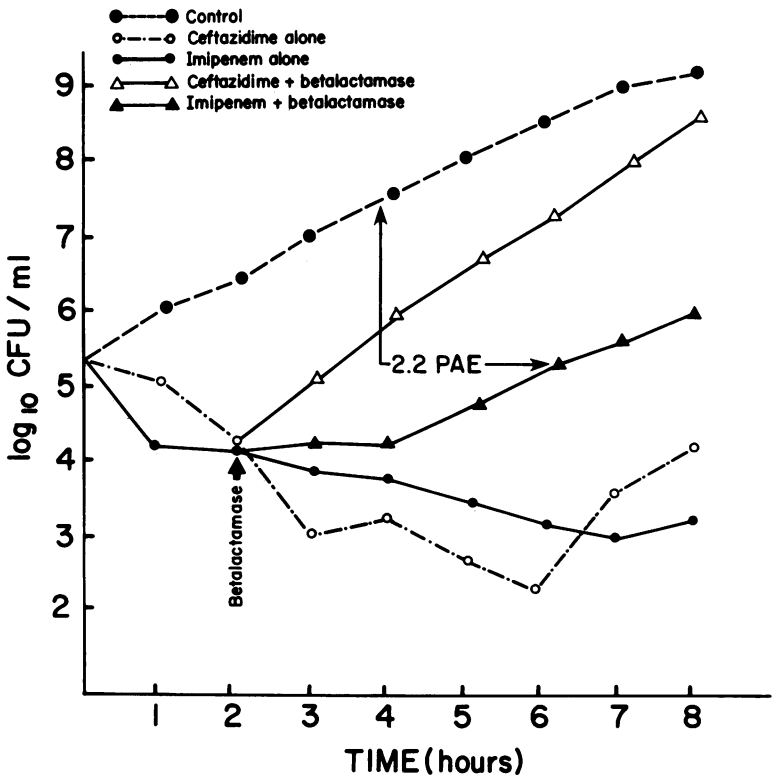

FIG. 3. *P. aeruginosa* strain 228 exposed for 2 h to imipenem and ceftazidime. Drug removal was effected by β-lactamase addition. Ceftazidime caused a negative PAE of 0.1 h.

in the design of more rational dosage schedules for these antimicrobial agents.

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