ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1980, p. 893-896
0066-4804/80/12-0893/04$02.00/0

Vol. 18, No. 6

Influence of Inoculum Size on Activity of Cefoperazone, Cefotaxime, Moxalactam, Piperacillin, and *N*-Formimidoyl Thienamycin (MK0787) Against *Pseudomonas aeruginosa*

MICHAEL L. CORRADO,[1]* SHELDON H. LANDESMAN,[1] AND CHARLES E. CHERUBIN[2]

Division of Infectious Disease, Downstate Medical School, University of New York, Brooklyn, New York 11203,[1] and Infectious Disease Service, Brooklyn Jewish Hospital, Brooklyn, New York 11213[2]

Forty clinical isolates of *Pseudomonas aeruginosa* were tested for their susceptibility to cefoperazone, cefotaxime, moxalactam, piperacillin, *N*-formimidoyl thienamycin (MK0787), and gentamicin at three different inocula. At an inoculum of 5×10^3 colony-forming units (CFU) per ml, the mimimum inhibitory concentrations (in micrograms per milliliter) for 90% of isolates (MIC_{90}) were as follows: gentamicin, 1; *N*-formimidoyl thienamycin, 2; cefoperazone, 4; piperacillin, 8; moxalactam, 16; and cefotaxime, 16. When the inoculum was increased to 5×10^5 CFU/ml, the MIC_{90} for all drugs tested increased. Among the beta-lactam antibiotics, *N*-formimidoyl thienamycin and cefoperazone had the lowest MIC_{90} (8 μg/ml) at this inoculum. When the inoculum was increased further to 5×10^7 CFU/ml, an MIC_{90} could be determined only for gentamicin and *N*-formimidoyl thienamycin (4 and 8 μg/ml, respectively). Indeed, the MIC_{50} for moxalactam, cefotaxime, cefoperazone, and piperacillin was 128 μg/ml or more at this inoculum. The minimum bactericidal concentration for 90% of isolates (MBC_{90}) at an inoculum of 5×10^5 CFU/ml ranged from 8 μg/ml for gentamicin and *N*-formimidoyl thienamycin to 128 μg/ml for cefotaxime. At the highest inoculum, however, whereas the MBC_{90} for gentamicin and *N*-formimidoyl thienamycin remained at 8 μg/ml, the MBC_{90} for each of the other drugs was >128 μg/ml. *N*-Formimidoyl thienamycin was the only drug tested for which an MIC_{100} and MBC_{100} (MIC and MBC for 100% of isolates) could be determined, and these were not significantly different from the MIC_{90} and MBC_{90}.

A number of recently developed beta-lactam antibiotics, including piperacillin (2), cefoperazone (5), cefotaxime (9), moxalactam (1), and the structurally novel *N*-formimidoyl thienamycin (MK0787) (6), have displayed significant activity against *Pseudomonas aeruginosa* in vitro and in this respect are clearly different from the currently available representatives or other recently introduced members of this compound class. Since the activities of beta-lactam antibiotic derivatives are usually impaired by large inocula, it seemed important to determine whether all of the agents that exhibited this remarkable activity against *P. aeruginosa* share this liability or whether some more closely resemble those aminoglycosides whose activity is not influenced by large inocula. The results of such a study are recorded in this report.

MATERIALS AND METHODS

Organism. Forty conventionally characterized (4) isolates of *P. aeruginosa* from the blood or urine of patients at the Kings County and Brooklyn Jewish hospitals were used in this study. All isolates were maintained on nutrient agar slants (Scott Laboratories, Inc.) at 25°C until used.

Antibiotics. Stock solutions of gentamicin (Schering Corp.), cefotaxime (Hoechst-Roussel Pharmaceuticals, Inc.), piperacillin (Lederle Laboratories), and cefoperazone (Pfizer Inc.) in sterile water (6,400 μg/ml) and moxalactam (Eli Lilly & Co.) in 1 M phosphate buffer (pH 7; 2,560 μg/ml) were stored at −70°C until used. Because of uncertainties as to the stability of the compounds, stock solutions of *N*-formimidoyl thienamycin in sterile water (2,560 μg/ml) were prepared daily.

Susceptibility testing. Susceptibility testing by both broth dilution and microtiter broth dilution procedures was carried out in Mueller-Hinton broth (MHB; BBL Microbiology Systems) which was supplemented with 5 mg of Ca^{2+} per dl and 2.5 mg of Mg^{2+} per dl for tests with gentamicin. Each isolate was grown at 37°C in MHB for 6 h. Culture populations were adequate to approximately 10^8 colony-forming units (CFU) per ml by comparison with a 0.5 McFarland standard. Serial 200-fold dilutions in MHB were prepared to give suspensions containing 10^6 and 10^4 CFU/ml. Final inocula for microtiter broth dilution tests were prepared by adding 50 μl of the above suspensions to each well of a microtiter plate that contained serial twofold dilutions of the appropriate

antibiotic in 50 μl of MHB. The final volume of each well was 100 μl. Final inocula for susceptibility testing were therefore 5×10^7, 5×10^5, and 5×10^3 CFU/ml. To verify these numbers, total counts were performed on each set of inocula by subculture on Mueller-Hinton agar (MHA; BBL). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of antibiotic which prevented visible growth after overnight culture at 37°C. The minimum bactericidal concentration (MBC) was determined by using the Dynatech MIC 2000 inoculator by transferring 1.5 μl of inoculum from each well to an MHA surface. The MBC for the microtiter system was defined as the lowest concentration of antibiotic which upon subculture failed to yield growth from wells which received an inoculum of 5×10^7 CFU/ml. For wells which received inocula of 5×10^3 and 5×10^5 CFU/ml, the MBC was determined by subculturing the entire contents of each well containing no visible growth onto MHA. The MBC was defined for an inoculum of 5×10^5 CFU/ml as the lowest concentration of antibiotic which produced a 99.9% reduction in CFU per milliliter when compared with growth controls for each inoculum used. Since a 99.9% killing could not be determined for an inoculum of 5×10^3 CFU/ml, the MBC was arbitrarily defined as the lowest concentration of antibiotic which upon subculture of the contents of the well onto MHA yielded no more than one colony.

In the macrotiter broth dilution tests, performed to confirm the results of the microtiter tests, growth suspensions were prepared as described above with a 0.5 McFarland standard. To each tube containing 0.5 ml of twofold serially diluted antibiotic, 0.5 ml of each suspension of organisms was added. The final volume in each tube was therefore 1.0 ml, and the final inocula were 5×10^7, 5×10^5, and 5×10^3 CFU/ml. The MIC was defined as the lowest concentration of antibiotic which failed to yield visible growth after overnight incubation at 37°C. The MBC for the broth dilution method was determined for each inoculum by subculturing 100 μl from each tube which failed to support visible growth onto MHA. The MBC for this system was defined as the lowest concentration of antibiotic which yielded a 99.9% reduction in CFU when compared with growth controls for each inoculum.

RESULTS

The MICs and MBCs obtained by both the broth microtiter and macrotiter systems were usually identical or no more than twofold different, in which case the microtiter system typically gave the lower MIC or MBC. Since the MICs and MBCs obtained by the microtiter system did not differ by more than twofold from those obtained by the macrotiter system, only results obtained with the microtiter method will be referred to here. At an inoculum of 5×10^3 *N*-formimidoyl thienamycin was the most active of the beta-lactam antibiotics, with 90% of isolates requiring an MIC (MIC_{90}) of 2 μg/ml or less as compared with 4 μg/ml for cefoperazone, 8 μg/ml for piperacillin, and 16 μg/ml for cefotaxime and moxalactam (Table 1). The MIC_{90} of all five beta-lactam antibiotics increased as the inoculum was increased from 5×10^3 to 5×10^7 CFU/ml. This increase, however, was least with *N*-formimidoyl thienamycin (MIC_{90}, 8.0 μg/ml [Table 1]). The MIC_{90} of gentamicin was virtually the same at inocula of 5×10^3 and 5×10^5 CFU/ml. With an increase in the MICs for piperacillin, moxalactam, cefotaxime, and cefoperazone, only an occasional isolate required an MIC of less than 128 μg/ml (Table 1). With 33 of the 40 isolates, there was an almost fourfold increase in the MIC of *N*-formimidoyl thienamycin as the inoculum was increased from 5×10^3 to 5×10^7 CFU/ml. The MICs for the remaining seven strains, however, did not exceed 8 μg/ml.

The MBCs of all six drugs were either identical to or only twofold higher than the MICs at an inoculum of 5×10^3 CFU/ml (Tables 1 and

TABLE 1. *Influence of inoculum size on concentrations of gentamicin, N-formimidoyl thienamycin, moxalactam, cefotaxime, and piperacillin required to inhibit growth of 40 isolates of P. aeruginosa by a broth microtiter system*

Compound	MIC (μg/ml of medium)								
	Range			For % of isolates					
				50			90		
	5×10^3[a]	5×10^5	5×10^7	5×10^3	5×10^5	5×10^7	5×10^3	5×10^5	5×10^7
Gentamicin[b]	0.06–64	0.125–64	0.5–128	0.25	0.5	1	1	2	4
N-Formimidoyl thienamycin	0.06–8	0.25–16	0.5–16	0.5	1	4	2	8	8
Moxalactam	0.5–32	0.5–64	16–>128	4	8	128	16	32	>128
Cefotaxime	1–64	4–128	16–>128	4	8	>128	16	32	>128
Cefoperazone	0.5–16	1–32	16–>128	2	4	>128	4	8	>128
Piperacillin	0.5–16	2–64	32–>128	2	4	>128	8	16	>128

[a] Inoculum size expressed as CFU per milliliter.
[b] Based on 38 susceptible isolates.

TABLE 2. *Influence of inoculum size on concentrations of gentamicin, N-formimidoyl thienamycin, moxalactam, cefotaxime, cefoperazone, and piperacillin required for bactericidal activity for 40 isolates of P. aeruginosa by a broth microtiter system*

Compound	MBC (μg/ml of medium)								
	Range			For % of isolates:					
				50			90		
	5×10^{3a}	5×10^{5}	5×10^{7}	5×10^{3}	5×10^{5}	5×10^{7}	5×10^{3}	5×10^{5}	5×10^{7}
Gentamicin[b]	0.06–128	0.25–128	0.5–128	0.5	1	2	1	8	8
N-Formimidoyl thienamycin	0.25–8	0.5–16	1.0–16	1	2	4	4	8	8
Moxalactam	0.5–64	4–64	16–>128	8	16	>128	32	32	>128
Cefotaxime	1–128	8–>128	64–>128	8	16	>128	32	128	>128
Cefoperazone	0.5–32	1–>128	32–>128	2	4	>128	8	64	>128
Piperacillin	1–16	2–>128	64–>128	2	4	>128	16	32	>128

[a] Inoculum size expressed as CFU per milliliter.
[b] Based on 38 susceptible isolates.

2). This close relationship between the MIC and MBC for *N*-formimidoyl thienamycin remained constant throughout the ranges of inocula tested. There were fourfold and eightfold spreads between the MICs and MBCs of cefotaxime and cefoperazone at an inoculum of 5×10^5 CFU/ml, respectively (Tables 1 and 2). The dimensions of the divergence of the MBC and MIC at an inoculum of 5×10^7 could not be determined for piperacillin, cefotaxime, cefoperazone, and moxalactam since neither MIC nor MBC endpoints were obtained with most isolates.

It was interesting to note that only *N*-formimidoyl thienamycin exhibited an MIC_{100} or MBC_{100} at a concentration of 16 μg/ml at an inoculum of 5×10^7 CFU/ml. The MIC_{100} of all other drugs, including gentamicin, was 64 μg/ml or more, even at an inoculum of 5×10^5 CFU/ml.

DISCUSSION

Carbenicillin was the first of the beta-lactam antibiotics to exhibit significant activity against *P. aeruginosa*. The activity of carbenicillin against some isolates of this organism was marginal and was regularly reduced by inocula of 10^6 CFU/ml or greater (1).

The newer, broad-spectrum beta-lactam antibiotics cefotaxime, cefoperazone, moxalactam, and piperacillin offer a wide spectrum and are more active than carbenicillin against *P. aeruginosa* (1, 2, 5, 7, 9). However, like carbenicillin, the activities of piperacillin (2, 8), cefotaxime (1), and moxalactam (1), as well as of azlocillin and mezlocillin (3), two other new beta-lactam antibiotics, are reduced with increases in inoculum size. Our data confirm these findings for piperacillin, moxalactam, and cefotaxime and also show that such an effect exists for cefoperazone. At an inoculum of 5×10^7 CFU/ml, virtually all isolates tested had an MIC well above the therapeutic range of these four drugs (Table 1).

N-Formimidoyl thienamycin was as active or more active than the other beta-lactam antibiotics at an inoculum of 5×10^3 CFU/ml. This compound differed significantly, however, from the other beta-lactam antibiotics in that its activity against *P. aeruginosa* was not affected by increases in inoculum size. For the few isolates which exhibited more than a fourfold rise in MICs when the inoculum was increased, *N*-formimidoyl thienamycin was still effective at 8 μg/ml or less at an inoculum of 5×10^7 CFU/ml, a concentration which is attainable in the serum (Merck Sharp & Dohme, unpublished data). At no inoculum was there a great difference between the MIC and MBC of *N*-formimidoyl thienamycin. In this respect, this thienamycin derivative resembled gentamicin more than it resembled other beta-lactam antibiotics.

ACKNOWLEDGMENTS

We thank Michael Shulman, Yassim Mohamed, Maharanee Jhagroo, Andrea Gruarin, and Marinella Cummings for their technical help. We also thank Marie Torlone for her assistance in the preparation of the manuscript.

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