Pirbenicillin: Comparison with Carbenicillin and BL-P1654, Alone and with Gentamicin, Against *Pseudomonas* *aeruginosa*

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Minimum inhibitory concentrations (MIC) of pirbenicillin against 135 clinical isolates of *Pseudomonas aeruginosa* were one-fourth of those required for carbenicillin but two times higher than those for BL-P1654. Increasing the inoculum size produced an adverse effect on the bactericidal activity for all three antibiotics. This was more apparent for pirbenicillin than for carbenicillin, but less than the effect on BL-P1654. When concentrations of antibiotics likely to be achieved clinically were used, gentamicin increased the inhibitory and bactericidal effects of all three semisynthetic penicillins for the majority of isolates. Strains highly resistant to the aminoglycoside antibiotic, however, were inhibited no more by the penicillin-gentamicin combinations than by the most effective of the antibiotics alone.

Pirbenicillin, previously known as CP-33,994-2, is a new semisynthetic penicillin with a spectrum of antibacterial activity similar to that of carbenicillin. It is reported to have greater potency against *Pseudomonas aeruginosa*, *Streptococcus faecalis*, and *Haemophilus influenzae* than carbenicillin but is less active against *Proteus* species (3, 22). The present study compares the in vitro activity of this new penicillin with that of carbenicillin and BL-P1654 against *P. aeruginosa*. BL-P1654 is also a penicillin with activity against *P. aeruginosa* (14, 20, 21, 24, 28, 29) and has been found to have good inhibitory but poor bactericidal activity against these organisms. It was included in this study because it has a chemical structure somewhat similar to that of pirbenicillin (Fig. 1).

MATERIALS AND METHODS

One hundred and thirty-five clinical isolates of *P. aeruginosa* were obtained from the Baltimore Veterans Administration Hospital, Baltimore Cancer Research Center (National Cancer Institute), and the University of Maryland Hospital. Some of the organisms were selected because they were resistant to carbenicillin or gentamicin by routine Kirby-Bauer disk susceptibility testing. Cultures were maintained on Trypticase soy agar (BBL, Cockeysville, Md.) at -70°C until studied, passed twice in Trypticase soy broth prior to testing to produce a luxuriant growth, and plated onto blood agar to insure purity. All comparative studies were performed in parallel in Trypticase soy broth at a pH of approximately 7.3.

The antibiotics, in concentrations of 25,000 $\mu\text{g/ml}$ for pirbenicillin and carbenicillin and 1,000 $\mu\text{g/ml}$ for BL-P1654 and gentamicin, were freshly prepared once a week in Trypticase soy broth and frozen until used. Pirbenicillin and carbenicillin were kindly furnished by Pfizer Laboratories; BL-P1654 and gentamicin were from Bristol Laboratories and the Schering Corp., respectively.

Antibiotic susceptibility studies. The minimum inhibitory concentrations (MIC) of the three penicillins alone and in combination with gentamicin were determined by a microtiter modification of the checkerboard technique, as previously described by this laboratory (12). Utilizing this method, minimum inhibitory concentrations were obtained using twofold dilutions of each penicillin alone, twofold dilutions of gentamicin alone, and each penicillin in combination with gentamicin at every dilution. The inoculum was 0.1 ml of a 10^{-3} dilution of an overnight culture added to 0.1 ml of broth containing the antibiotics. The MIC was the lowest concentration that prevented grossly detectable growth after 18 h of incubation at 37°C . Previous studies have indicated that the MIC obtained by this method is almost identical to that obtained by the conventional macrotube technique (12).

Minimum bactericidal concentrations (MBC) were determined for 58 carbenicillin-susceptible strains. These strains were selected at random from a pool of strains with an MIC of carbenicillin of less than, or equal to, 100 $\mu\text{g/ml}$. Because of the difficulty in subculturing from microtiter wells, the bactericidal studies were determined by the conventional macrotube technique, using an inoculum of 0.5 ml of a 10^{-3} dilution of an overnight culture, added to the same volume of broth containing twofold dilutions of the antibiotics. After 18 h of incubation

FIG. 1. Chemical structure of pirbenicillin, carbenicillin, and BL-P1654.

tion, the inhibitory end points were determined, and the tubes without visible growth were subcultured onto blood agar plates with a 0.01-ml calibrated loop. The MBC was defined as the lowest concentration that resulted in growth of less than 10 colonies (approximately 99.9% killing of the inoculum).

The influence of the inoculum size on the inhibitory and bactericidal end points was determined in a similar manner. The inocula were 0.5 ml of 10^{-2} , 10^{-4} , and 10^{-5} dilutions of an overnight culture and 0.5 ml of the standard 10^{-8} dilution.

The bactericidal effect of adding gentamicin to the three penicillins was determined in a total volume of 2 ml, which contained twofold dilutions of a penicillin, a final subbactericidal concentration of 1.2 μg of gentamicin per ml, and 1 ml of the standard inoculum. Subculturing for bactericidal end points was performed as described above from tubes containing the penicillin-gentamicin combination as well as each antibiotic alone.

The rates of killing of seven carbenicillin-susceptible strains of *P. aeruginosa* (chosen at random from strains with MICs of carbenicillin of less than or equal to 100 $\mu\text{g}/\text{ml}$) by 200 μg of the three penicillins per ml were determined in parallel. The MICs of carbenicillin for six of the seven strains tested were 50 $\mu\text{g}/\text{ml}$, and one strain was inhibited by 100 $\mu\text{g}/\text{ml}$. For pirbenicillin, three strains had an MIC of 25 $\mu\text{g}/\text{ml}$ and four were inhibited by 12.5 $\mu\text{g}/\text{ml}$. BL-P1654 inhibited three strains at 12.5 $\mu\text{g}/\text{ml}$ and four

at a concentration of 6.25 $\mu\text{g}/\text{ml}$. The inoculum was 2 ml of a 10^{-2} dilution of an overnight culture in a total volume of 20 ml. Colony counts were obtained by sampling 0.1 ml at 0, 4, 8, and 24 h, diluting appropriately in buffered saline, and subculturing into melted Trypticase soy agar. The number of colonies representing viable bacteria was counted after 18 h of incubation at 37°C. After the broth was filtered to remove the surviving bacteria, the residual antibiotic in the tubes was assayed by a modified agar well diffusion method, previously described (1), using *P. aeruginosa* NTCC 10701 as the assay organism. Residual antibiotic was also measured in control tubes containing antibiotic and broth, but no organisms, which were incubated in an identical fashion.

RESULTS

MICs. The MICs of each of the three penicillins alone for all 135 isolates of *P. aeruginosa* are shown in Fig. 2. The MICs of pirbenicillin were one-fourth the inhibitory concentrations of carbenicillin but two times higher than those of BL-P1654. Approximately 80% of the strains could be inhibited by 12.5 μg of BL-P1654, 25 μg of pirbenicillin, and 100 μg of carbenicillin per ml. The cross-susceptibility between pirbenicillin and carbenicillin is seen in Table 1. Strains susceptible or moderately resistant to carbenicillin (MIC $\leq$ 200 $\mu\text{g}/\text{ml}$) were susceptible to pirbenicillin, all but two being inhibited by 100 μg or less of this antibiotic per ml. However, all but one of the isolates with MICs of carbenicillin of 400 $\mu\text{g}/\text{ml}$ or greater were also resistant to 100 μg of pirbenicillin per ml. On the other hand, strains resistant to 100 μg of pirbenicillin per ml were also resistant to this concentration of carbenicillin. Similar results were obtained when comparing the cross-susceptibility of BL-P1654 and carbenicillin (Table 1).

MBCs. The MBCs and MICs of the three penicillins as performed by the conventional tube dilution technique against 58 carbenicillin-susceptible isolates of *P. aeruginosa* are expressed in Fig. 3. The strains studied were selected at random from strains with MICs of carbenicillin of 100 $\mu\text{g}/\text{ml}$ or less. In spite of the greater inhibitory activity of both pirbenicillin and BL-P1654 as compared to that of carbenicillin, the concentrations of these two drugs required to achieve killing were higher; 70% of strains required more than 400 $\mu\text{g}/\text{ml}$. Determinations of pH at the time of subculture from all clear tubes ranged from 7.3 to 7.4 for five of the six strains tested, and therefore a pH change of the medium did not accompany the diminished bactericidal effect.

Inoculum effect. The effect of the inoculum on the MIC and MBC for 10 carbenicillin-susceptible strains is seen in Fig. 4 and 5, respec-

FIG. 2. MICs of the three semisynthetic penicillins against 135 isolates of *P. aeruginosa*.

TABLE 1. Cross-resistance between carbenicillin, pirbenicillin, and BL-P1654 for 135 isolates of *P. aeruginosa*

Carbenicillin MIC	No. of isolates tested	No. of isolates inhibited by:									
		Pirbenicillin MIC (μ g/ml) of:					BL-P1654 MIC (μ g/ml) of:				
		≤ 25	50	100	200	≥ 400	≤ 25	50	100	200	≥ 400
≤ 100	108	100	6	2	0	0	104	2	2	0	0
200	17	4	9	2	2	0	14	1	2	0	0
≥ 400	10	0	0	1	1	8	0	1	0	3	6

tively. The marked effect of the inoculum on the inhibitory activity of all three antibiotics is apparent. When the inoculum was 10^6 bacteria/ml (our standard inoculum), pirbenicillin and BL-P1654 had greater inhibitory activity than carbenicillin. However, at 10^7 bacteria/ml, the MIC of all three antibiotics was generally greater than 400 μ g/ml. The adverse inoculum effect was more apparent for the bactericidal concentrations of the three drugs. With an inoculum of 10^4 bacteria/ml, all three penicillins appeared bactericidal, and both pirbenicillin and BL-P1654 were somewhat superior to carbenicillin. At an inoculum of 10^5 bacteria/ml, a standard inoculum in some laboratories, pirbenicillin was more bactericidal than carbenicillin, but BL-P1654 showed inferior killing against 6 of the 10 isolates. At 10^6 bacteria/ml, pirbenicillin and BL-P1654 were less bactericidal when compared to carbenicillin. The bactericidal activity of pirbenicillin was, therefore, adversely affected more than carbenicillin, but less than BL-P1654, by changes in the inoculum size.

Bactericidal rates. The comparative rates of killing of *P. aeruginosa* by 200 μ g of carbenicillin, pirbenicillin, and BL-P1654 per ml were determined in parallel for seven strains. During the first 8 h, the rates of killing of these strains were almost identical for the three antibiotics. By 24 h, however (Table 2), carbenicillin appeared slightly more cidal than the other two penicillins. Colony counts were less than 100 bacteria/ml for all of the strains exposed to carbenicillin but for only four of the seven strains in contact with pirbenicillin. BL-P1654 was the least cidal, with four of the seven strains having greater than 10^4 bacteria/ml at this time.

The percentage of antibiotic deterioration for all three antibiotics during the 24 h of incubation is seen in Table 3. Deterioration was slightly greater when bacteria were present as compared to the antibiotics in broth without bacteria (controls). Twenty percent of the initial concentration of pirbenicillin incubated with the bacteria and 16% of carbenicillin deteriorated over a 24-h period. Fifty-four percent of

FIG. 3. Comparative MICs and MBCs of the three penicillins for 58 carbapenem-susceptible isolates of *P. aeruginosa*.

FIG. 4. Effect of inoculum size (expressed as colony-forming units per milliliter) on the MIC of the three penicillins against 10 isolates of *P. aeruginosa*.

FIG. 5. Effect of inoculum size (expressed as colony-forming units per milliliter) on the MBC of the three penicillins against 10 isolates of *P. aeruginosa*.

TABLE 2. Comparative killing of seven strains of *P. aeruginosa* by 200 μ g of the three penicillins per ml at 24 h of incubation

Antibiotic	No. of strains with colony counts per ml at 24 h of:					
	0	1-10	11-100	101-1,000	1,001-10,000	>10,000
Carbenicillin	0	4	3	0	0	0
Pirbenicillin	0	0	4	1	2	0
BL-P1654	0	0	1	2	0	4

TABLE 3. Comparative percentage of antibiotic deterioration after 24 h of incubation at 37°C with and without (control) bacteria

Antibiotic	Antibiotic deterioration (%) in 24 h	
	With bacteria ^a	Control ^b
Carbenicillin	16 (10-18)	8
Pirbenicillin	20 (17-24)	12
BL-P1654	54 (52-59)	39

^a Average (range) for seven determinations.

^b One determination.

BL-P1654 deteriorated in the same time interval.

Although there was more deterioration of pirbenicillin and BL-P1654 compared to carbenicillin, the ratio of residual antibiotic at 24 h to the MIC for the strains tested was greater for pirbenicillin and BL-P1654. This residual antibiotic-to-MIC ratio was always greater than 6 for pirbenicillin and BL-P1654, whereas it was

less than 4 for carbenicillin. It is, therefore, unlikely that antibiotic deterioration alone is responsible for the differences in bactericidal activity among the three antibiotics.

Adding gentamicin to the penicillins. The activity of all three penicillins was enhanced by the addition of gentamicin. This is illustrated in Fig. 6, which compares the inhibitory concentrations of each of the penicillins alone and in combination with a subinhibitory concentration of 0.6 μ g of gentamicin per ml. The combination resulted in a greater inhibition (illustrated by a leftward shift of the MIC curves) by each of the penicillins as compared to each of the three drugs alone. In combination with gentamicin, the inhibitory activity of pirbenicillin remained superior to that of carbenicillin but inferior to that of BL-P1654. The addition of gentamicin likewise increased the bactericidal activity of the three penicillins (Fig. 7). The bactericidal effect produced by adding 1.2 μ g of gentamicin per ml (not bactericidal for the

FIG. 6. Comparison of the MICs of the three penicillins alone and in combination with a subinhibitory concentration of 0.6 μ g of gentamicin per ml against 135 isolates of *P. aeruginosa*.

FIG. 7. Comparison of the MBCs of the three penicillins alone and in combination with a subbactericidal concentration of 1.2 μ g of gentamicin per ml against 24 isolates of *P. aeruginosa*.

strains when used alone) to both pirbenicillin and BL-P1654 appeared somewhat superior to the effect of carbenicillin in combination with the aminoglycoside.

When concentrations likely to be achieved clinically were evaluated (up to 100 μg of each penicillin per ml and 5 μg of gentamicin per ml), the increased inhibitory effect of the penicillins resulting from adding gentamicin occurred for strains susceptible and/or moderately resistant to the aminoglycoside (Table 4). On the other hand, an increased inhibitory effect by the combination compared to the most effective antibiotic alone seldom occurred if the organism was highly resistant to the aminoglycoside antibiotic (MIC of gentamicin of ≥ 80 $\mu\text{g}/\text{ml}$) regardless of what penicillin was used in the combination. Only 12.5, 6.3, and 9.4% of these highly resistant strains showed an increased inhibitory effect when carbenicillin, pirbenicillin, and BL-P1654, respectively, were the semisynthetic penicillins in the combination.

DISCUSSION

Carbenicillin is highly effective against *P. aeruginosa* (2, 9, 16, 18, 25-27). Because of the great concentrations of carbenicillin required to inhibit these organisms, an undesirable sodium load with occasional circulatory congestion, frequent hypokalemia (11), occasional abnormalities of coagulation (4, 17), and, rarely, granulocytopenia (23) may be produced. Pirbenicillin, a monosodium salt, inhibits *P. aeruginosa* at concentrations that are generally one-fourth those of carbenicillin. Potentially, it may be clinically effective at lower doses and avoid some of these dose-related toxic effects.

With the standard tube dilution technique using 10^6 bacteria/ml as the inoculum, the concentrations of this compound required for cidal activity were much higher than those necessary for inhibition. The bactericidal end points for all three antibiotics were highly inoculum dependent, with the killing of *Pseudomonas* by pirbenicillin being more affected than that by carbenicillin but less than that by BL-P1654. Similarly, the rate of killing of *P. aeruginosa*

by pirbenicillin was less than that by carbenicillin but greater than that by BL-P1654. The reasons for this are not readily apparent. Pirbenicillin is reported to be unstable in an alkaline pH (22). Since the metabolic products of *P. aeruginosa* create an alkaline environment in unbuffered amino acid-containing media, it has been suggested that this pH change causes deterioration of the antibiotic, therefore allowing the organisms to proliferate. Supporting this, Bodey has found marked antibiotic deterioration in unbuffered Mueller-Hinton broth. The regrowth of organisms resulting from this deterioration was prevented by replenishing the antibiotic in the media (3). Similarly, Retsema et al. (22) have found the differences between inhibitory and cidal concentrations to be minimal in buffered brain heart infusion broth. The present study, however, was performed in buffered Trypticase soy broth. Significant pH changes did not occur. Although deterioration of pirbenicillin was greater than the deterioration of carbenicillin, it was not sufficient by itself to explain the discrepancy between the inhibitory and cidal activity. Residual pirbenicillin at 24 h was always at least six times the inhibitory concentration of the organisms. For carbenicillin, the residual antibiotic-to-MIC ratio was less than 4, yet the cidal activity of carbenicillin was greater. Other factors, alone or in addition to antibiotic deterioration, must be responsible for these differences. Perhaps intracellular destruction of the antibiotics by *Pseudomonas* may be greater than that noted in the broth. Such destruction would certainly be inoculum dependent and may be influenced by pH and media differences.

P. aeruginosa are opportunistic invaders in patients with compromised host defenses (5-7, 15, 19, 25, 26). Bactericidal antibiotics appear desirable for the treatment of these infections. Carbenicillin is known to exhibit good in vitro bactericidal activity against *P. aeruginosa* (8, 24, 29) and is of proven efficacy alone and combined with gentamicin in the treatment of *P. aeruginosa* infections in compromised hosts (2, 9, 10, 25, 26). Whether or not pirbenicillin will be more or less bactericidal compared to carben-

TABLE 4. Relationship of gentamicin susceptibility to the increased inhibitory effect produced by the three penicillin-gentamicin combinations in therapeutic concentrations against 135 strains of *P. aeruginosa*

Gentamicin MIC ($\mu\text{g}/\text{ml}$)	No. of strains	No. (%) of strains showing increased inhibition		
		Carbenicillin	Pirbenicillin	BL-P1654
≤ 5 , susceptible	89	89 (100)	89 (100)	89 (100)
10-40, moderately resistant	14	11 (76)	14 (100)	12 (86)
≥ 80 , highly resistant	32	4 (12.5)	2 (6.3)	3 (9.4)

^a Semisynthetic penicillin in the combination.

icillin in the clinical setting cannot be determined from these studies. However, careful observation is required to define this point if clinical trials are pursued.

Gentamicin clearly enhances the inhibitory and bactericidal ability of pirlbenicillin, as it does for the other antipseudomonal penicillins. Theoretically, the aminoglycoside-penicillin combination may reduce the emergence of resistant *P. aeruginosa* during therapy. These features lend support to the use of combined penicillin-aminoglycoside therapy in serious systemic infections caused by *P. aeruginosa* unless the organisms are found to be highly resistant to the aminoglycoside component of the combination (13).

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