Synergism of Azlocillin, Mezlocillin, Piperacillin in Combination with Tobramycin Against Klebsiella and Pseudomonas

JAMES T. DOWNS, B.S.,a VINCENT T. ANDRIOLE, M.D.,b AND JOHN L. RYAN, Ph.D., M.D.abc

aInfectious Disease Section, VA Medical Center, West Haven, Connecticut; bDepartment of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut

Received November 8, 1985

Fifty-three clinical isolates of Klebsiella and fifty-one clinical isolates of Pseudomonas aeruginosa, twenty-six of which were carbenicillin-(CARB) resistant, were tested for susceptibility to mezlocillin (MEZ), azlocillin (AZL), and piperacillin (PIP), both alone and in combination with tobramycin (TOB) using the microtiter broth diluent method and an inoculum density of 10⁶ CFU/ml. The Klebsiella were highly resistant to TOB, MEZ, and PIP (MIC₉₀: 8, > 256, > 128 μg/ml, respectively). Synergy was demonstrated in 53 percent (PIP/TOB) and 51 percent (MEZ/TOB). An indifferent response was observed in 47 percent (PIP/TOB) and 49 percent MEZ/TOB of the Klebsiella. PIP, MEZ, and AZL in combination with TOB showed synergism against CARB-resistant Pseudomonas in less than 10 percent of the strains tested. Synergy could be demonstrated against CARB-susceptible Pseudomonas with the combinations PIP/TOB, AZL/TOB, and MEZ/TOB in 12 percent, 12 percent, and 24 percent, respectively, of the twenty-five strains tested. Indifferent effects were observed in 84 percent, 88 percent, and 76 percent, respectively, of these same CARB-susceptible strains. These data suggest that there is no significant difference in the incidence of synergy with these new penicillins and tobramycin against either Pseudomonas or Klebsiella.

INTRODUCTION

Aminoglycosides, combined with semisynthetic penicillins, are the most widely studied antibiotic combinations for use against gram-negative bacilli, the major cause of serious sepsis in immunocompromised patients. Klebsiella and Pseudomonas represent two of the most significant pathogens in this patient population. The use of two or more antimicrobial agents for empiric therapy of such infections has become common in clinical practice [15] and is based on the hope, suggested by in vitro studies, that the drugs may interact synergistically to inhibit infecting pathogens. There are data from both animal and human trials that indicate improved clinical efficacy of synergistic antibiotic combinations [1,2,14].

New broad-spectrum penicillin derivatives have been synthesized which have potent activity against both Klebsiella and Pseudomonas. Indications for use of these agents are unclear. Piperacillin and azlocillin have enhanced activity against Pseudomonas, while piperacillin and mezlocillin have enhanced activity against Klebsiella compared to carbenicillin and ticarcillin. A critical parameter in the therapy of infections with Pseudomonas is the presence of synergy. This report compares the in vitro activity of...
three recently introduced semisynthetic penicillins (azlocillin, mezlocillin, and piperacillin) alone and in combination with the aminoglycoside, tobramycin, against clinical isolates of Klebsiella species and of Pseudomonas aeruginosa to see if there is a clear advantage with respect to synergy for any of the penicillins. Several investigators have reported that azlocillin shows reduced efficacy against active β-lactamase producing strains such as Klebsiella [4,5]. Therefore, azlocillin was not tested against Klebsiella sp. in this study.

MATERIALS AND METHODS

Bacterial Strains

Fifty-three Klebsiella species and 51 Pseudomonas aeruginosa strains were collected as unique isolates from the microbiology laboratories of Yale–New Haven Hospital, New Haven, CT, the Veterans Administration Medical Center, West Haven, CT, and the Hospital of St. Raphael, New Haven, CT. Initially, the minimum inhibitory concentration (MIC) of carbenicillin for each Pseudomonas strain was tested in duplicate with the microtiter method outlined below. Strains having MICs less than 128 μg/ml (25/51) were taken as carbenicillin-susceptible; those with MICs above this level (26/51) were defined as carbenicillin-resistant.

Antibiotics

Antimicrobial agents were generously provided as standard powders or solutions by their manufacturers: tobramycin by Eli Lilly, Co., Indianapolis, IN; azlocillin and mezlocillin by Miles Pharmaceuticals, West Haven, CT; piperacillin by Lederle Laboratories, Pearl River, NY; carbenicillin by Roerig, New York, NY.

Susceptibility Testing

A single lot of Mueller-Hinton broth was used in all experiments (Difco, No. 707919). Solid medium was prepared by adding agar to a concentration of 15 percent to MHB. This lot of MHB contained 0.1 mg/ml of calcium and 0.02 mg/ml of magnesium. Thus both solid and liquid medium contained the same concentration of calcium and magnesium. The media were not supplemented with additional divalent cations. Standard Kirby Bauer disc susceptibility tests were performed using Pseudomonas aeruginosa, strain ATCC No. 27853, with azlocillin (75 μg), mezlocillin (75 μg), piperacillin (100 μg), and tobramycin (10 μg) impregnated discs. The diameters of zones of inhibition for all discs fell within normal ranges, confirming the quality of the solid medium.

MICs, minimum bactericidal concentrations (MBCs), and synergy were determined in microtiter plates with 96 U-shaped wells (Dynatech, Alexandria, VA) containing the antibiotics diluted in Mueller-Hinton broth (MHB). For the MICs, 100 μl of twofold serial dilutions of each antibiotic were added to each well. One well in each row contained only MHB to serve as an inoculation/growth control. Bacteria were grown overnight, then diluted in fresh broth to a density of approximately 1 × 10^8 CFU/ml, as determined with a spectrophotometer. A semiautomatic inoculator (Dynatech, Alexandria, VA) was used to deliver 1 × 10^5 CFU (1 × 10^6 CFU/ml) to each well (final concentration: 1 × 10^6 CFU/ml). The MIC was taken as the lowest concentration of drug with no visible growth after 18 hours of incubation at 37°C.

MBC data were obtained by inoculating Mueller-Hinton agar plates with an aliquot (1.5 μl) from each well of the MIC plate followed by incubation at 37°C for 48 hours.
No growth was required to define the MBC (>99.9 percent killing). Thus in the absence of killing, an aliquot would contain at least $1.5 \times 10^3$ CFUs. At 99.9 percent killing this aliquot would contain 1.5 CFUs which would be detectable by agar plating.

Antibiotic synergy testing employed the same microtiter broth method described above except that dilutions of tobramycin were placed horizontally in the trays, while those of each penicillin were placed vertically, resulting in a checkerboard array of drug combinations. The concentrations chosen for each drug insured that a range from one-eighth to two times the MIC for all but very resistant strains was included. The same inoculum was delivered to each well, one well per row serving as drug-free control. After incubation at 37°C for 24 hours, the lowest concentration of drugs showing no visible growth was taken as the best combination.

**Synergy Criteria**

Synergy data were evaluated using published methods [8,13] by which the fractional inhibitory concentrations (FICs) and FIC indexes (FIXs) were calculated. The FIC for a single antibiotic was determined as the ratio of the MIC of that drug in combination, divided by the MIC when used alone. Thus, two FICs were calculated for each effective combination. The FIX was calculated as the numerical sum of the two FICs for a given combination. The following criteria were used: FIX less than or equal to 0.5, synergy; FIX greater than 0.5 but less than or equal to 2.0, indifferent effects; FIX greater than 2.0, antagonism. Typical equations for these calculations are shown:

$$FIC_x = \frac{MIC_x \text{ in combination}}{MIC_x \text{ alone}}$$

$$FIX = FIC_x + FIC_y$$

**RESULTS**

Figure 1 shows the cumulative distribution of the MICs of bacteria tested against the semisynthetic penicillins. In each case, carbenicillin-susceptible *Pseudomonas* were slightly more susceptible than the carbenicillin-resistant strains. Azlocillin and piperacillin appeared more active against *Pseudomonas* than mezlocillin, and piperacillin demonstrated more activity against the carbenicillin-resistant *Pseudomonas* than either of the other two drugs. The activities of mezlocillin and piperacillin against *Klebsiella* appeared to be similar.

The MICs and MBCs which were effective against 50 percent and 90 percent of the *Klebsiella* and *Pseudomonas* strains tested are shown in Table 1. As can be seen, some strains of *Klebsiella* were highly resistant to both mezlocillin and piperacillin, the MIC$_{90}$ exceeding the highest drug concentrations used. The MIC$_{90}$ data for azlocillin, mezlocillin, and piperacillin against the two groups of *Pseudomonas* show that these drugs have similar anti-pseudomonal activities within groups: a twofold range for carbenicillin-resistant *Pseudomonas* (64–128 µg/ml); a fourfold range for carbenicillin-susceptible *Pseudomonas* (8–32 µg/ml). *Pseudomonas* strains resistant to carbenicillin show higher MICs for the three semisynthetic penicillins than those of the carbenicillin-susceptible strains.

Table 2 contains a summary of data from synergy testing. The mezlocillin-tobramycin combination synergistically inhibited 51 percent and had an indifferent
FIG. 1. The cumulative distribution of MICs for all of the strains tested are shown. The upper curves show the activity of azlocillin against carbenicillin-susceptible (-•-) and carbenicillin-resistant (-○-) Pseudomonas aeruginosa. The middle and lower curves show the same data for mezlocillin and piperacillin, respectively. In addition, the cumulative distribution of MICs of these latter two drugs against Klebsiella (-○-) is shown.

effect on 49 percent of the Klebsiella strains. With these same strains, piperacillin-tobramycin showed synergism against 53 percent and indifferent effects against 47 percent. The three penicillins each in combination with tobramycin showed relatively little synergism against either group of Pseudomonas strains. Mezlocillin-tobramycin showed the highest degree of synergy (24 percent) against carbenicillin-susceptible Pseudomonas. Indifferent effects for each penicillin-tobramycin combination were seen in 100 percent (mezlocillin-tobramycin), 80 percent (azlocillin-tobramycin), and 96 percent (piperacillin-tobramycin) of the carbenicillin-resistant strains, and 76 percent, 84 percent, and 84 percent, respectively, against the carbenicillin-susceptible

| TABLE 1 |
|---------|
| MICs/MBCs of Antibiotics Alone vs. Strains Tested |

|          | MIC (mcg/ml) | MBC (mcg/ml) |
|----------|--------------|--------------|
|          | Range  50% 90% | Range  50% 90% |
| Klebsiella (53) | MEZ 2->256 16 >256 | 2->256 32 >256 |
|           | PIP 1->128 32 128 | 1->128 64 >128 |
|           | TOB 0.6->8 0.5 >8 | 0.6->8 0.5 >8 |
| Pseudomonas (26) | AZL 2->512 16 64 | 4->512 32 >512 |
| (CARB-resistant) | MEZ 16->256 32 128 | 16->256 64 >256 |
|           | PIP 4->64 8 64 | 4->128 16 64 |
|           | TOB 0.06->8 0.12 4 | 0.06->8 0.25 >8 |
| Pseudomonas (25) | AZL 2->16 8 16 | 4->64 8 16 |
| (CARB-susceptible) | MEZ 4->64 16 32 | 16->128 16 64 |
|           | PIP 2->16 4 8 | 4->16 4 16 |
|           | TOB 0.06->8 0.12 1 | 0.06->8 0.25 >8 |

MEZ, mezlocillin; PIP, piperacillin; AZL, azlocillin; CARB, carbenicillin; TOB, tobramycin
organisms. Antagonism was observed in three strains of carbenicillin-resistant *Pseudomonas* tested with azlocillin-tobramycin, and in one strain of carbenicillin-susceptible *Pseudomonas* using piperacillin-tobramycin.

**DISCUSSION**

Heineman and Lofton [9] tested the *in vitro* response of *Pseudomonas* to penicillin-aminoglycoside combinations and concluded that an organism’s MIC for an individual drug gave no hint of its response to drug combinations, and that no particular combination of drugs could be used as a screen for other combinations. With this unpredictability assumed, this investigation was designed to determine the *in vitro* efficacy of tobramycin combined with each of three new semisynthetic penicillins. This study was designed to use a high inoculation density (1 x 10^6 CFU/ml) [5], to include carbenicillin-resistant organisms, and to adhere to strict synergy criteria in an attempt to evaluate these drugs under “most difficult” conditions of *in vitro* testing.

The analytical scheme used to assess synergy employs ratios and sums of ratios of drug concentrations. Investigators using this method have cited synergism with FIXs ranging from 0.3 to 0.75 [10,13]. Using the less strict criteria (FIX less than or equal to 0.75), the incidence of synergism in this study would be considerably increased. However, the criteria used in this report (FIX less than or equal to 0.5) allowed direct comparison of data to previous reports by authors defining synergy as a fourfold reduction of the MICs of both drugs in a combination [1,3,11]. In fact, when our data were calculated using a fourfold reduction of the MIC for both drugs as the synergy criteria, the results were identical.

Azlocillin, mezlocillin, and piperacillin when combined with tobramycin were shown in this study to have similar synergistic activities. Other investigators report higher levels of synergism (range: 32–100 percent) for penicillin-aminoglycoside pairs [3,10,12], particularly piperacillin-gentamicin [3,6]. Although differences in technique (e.g., test method, inoculum density, analysis criteria) may account for variability, penicilllase activity (or lack of it) is also important in the response of any individual strain. Other investigators have used β-lactamase producing and non-producing organisms when studying penicillin-derived drugs and have reported multi-modal distributions of MICs with penicillin-derived drugs [5,12], suggesting sub-populations of destructive enzyme producers. The trimodal distribution of *Kleb-
siella MICs (Fig. 1) may reflect the presence of β-lactamase production in some strains analyzed in this study. Also it has recently been shown [7] that selected semisynthetic penicillins when incubated with Enterobacteriaceae species are often inactivated after seven hours, presumably due to β-lactamase activity. The clinical significance of this inactivation is not clear.

These data do not demonstrate an advantage in the amount of synergy achieved with any of the combinations tested. Since it has been shown that the presence of antibiotic synergy represents an important parameter in predicting a positive response in *Pseudomonas* infections, these data may suggest there will be no clear advantage of any one of these combinations over another.

ACKNOWLEDGEMENTS

We thank James Layman for his excellent assistance and Karen Marino for preparing the manuscript. This work was supported, in part, by Miles Laboratories, West Haven, CT, and Eli Lilly & Co., Indianapolis, IN.

REFERENCES

1. Andriole VT: Antibiotic synergy in experimental infection with *Pseudomonas* II. The effect of carbenicillin, cephalothin or cephane combined with tobramycin or gentamicin. J Infect Dis 129:124–133, 1974
2. Andriole VT: Aminoglycoside antibiotics: Antibacterial efficacy in animal models of infection. Rev Infect Dis 5 (Suppl 2): S233–S249, 1983
3. Bach VT, Webb DW, Thadepalli H: Antimicrobial synergism of piperacillin and gentamicin against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus fecalis*. Chemotherapy 26:21–27, 1980
4. Barry AL, Thornsberry C, Jones RN, Gavan TL: *In vitro* activity of mezlocillin and azlocillin compared with that of four other penicillins and two aminoglycosides. Cleve Clin Q 47:311–319, 1980
5. Fu KP, Neu HC: Azlocillin and mezlocillin: new ureido penicillins. Antimicrob Agents Chemother 13:930–938, 1978
6. Fu KP, Neu HC: Piperacillin, a new penicillin active against many bacteria resistant to other penicillins. Antimicrob Agents Chemother 13:358–367, 1978
7. Glew RH, Pavuk RA: Early synergistic interaction between semisynthetic penicillins and aminoglyco-
sidic aminocyclitolts against Enterobacteriaceae. Antimicrob Agents Chemother 23:902–906, 1983
8. Hallander HO, Darnbusch K, Gezelius L, Jacobson K, Karlsson I: Synergism between aminoglycosides and cephalosporins with antipseudomonal activity: interaction index and killing curve method. Antimicrob Agents Chemother 22:743–752, 1982
9. Heineman HS, Lofton WM: Unpredictable response of *Pseudomonas aeruginosa* to synergistic antibiotic combinations *in vitro*. Antimicrob Agents Chemother 13:827–831, 1978
10. Hoogkamp-Korstanje JAA, Westerdall NAC: Activity and synergy of ureido penicillins and aminogly-
cosides against *Pseudomonas aeruginosa*. Infection 10 (Suppl 3): S257–S261, 1982
11. McLaughlin FJ, Matthews WJ, Streider PJ, Sullivan B, Taneja A, Murphy P, Goldman DA: Clinical and bacteriological responses to three antibiotic regimens for acute exacerbations of cystic fibrosis: ticarcillin-tobramycin, azlocillin-tobramycin, and azlocillin-placebo. J Infect Dis 147:559–567, 1983
12. Neu HC, Fu KP: Synergy of azlocillin and mezlocillin combined with aminoglycoside antibiotics and cephalosporins. Antimicrob Agents Chemother 13:813–819, 1978
13. Perea E, Torres M, Borobio M: Synergism of fosfomycin-ampicillin and fosfomycin-chloramphenicol against *Salmonella* and *Shigella*. Antimicrob Agents Chemother 13:705–709, 1978
14. Rahal JJ: Antibiotic combinations. Medicine 57:179–195, 1978
15. Reyes MW, Brown W, Lerner A: Treatment of patients with *Pseudomonas* endocarditis with high dose aminoglycoside and carbenicillin therapy. Medicine 57:57–68, 1978