PIPERACILLIN (T-1220), A NEW SEMISYNTHETIC PENICILLIN. II

IN VITRO ANTIMICROBIAL ACTIVITY AND SYNERGY COMPARISON WITH CARBENICILLIN AND GENTAMICIN

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Piperacillin, a new semisynthetic penicillin, was found to have potent antimicrobial activity against nearly all (405) tested bacterial species. Piperacillin was consistently 4~16-fold more active than carbenicillin against the Enterobacteriaceae, 16~32-fold against *Pseudomonas aeruginosa* and *Pseudomonas* species, and 16-fold against *Streptococcus faecalis*. Carbenicillin and piperacillin were equally effective against *Staphylococcus aureus*, but inactivated by beta-lactamase. A 38% overall synergy rate for the piperacillin-gentamicin combination was identified, a finding similar to that for carbenicillin-gentamicin. Highest incidences of synergy were found for both antibiotic pairs tested on gentamicin-resistant isolates (*Ps. aeruginosa* and *Providencia*).

In vitro findings suggest that piperacillin alone or in combination with aminoglycosides may be highly efficacious in the treatment of most serious bacterial infections.

Piperacillin, the recently described piperazine derivative of α-aminobenzyl penicillin has superior *in vitro* activity to similar semisynthetic penicillins and related beta-lactam antimicrobics[1~5]. The fact that beta-lactam antibiotics possess generally superior bactericidal and toxicity characteristics compared to other antimicrobial classes, *e.g.* aminoglycosides, prompted our group to extend earlier investigations[2,5]. Combinations of beta-lactam drugs and aminoglycosides have proven efficacy against a wide variety of organisms *in vitro*[6~8] as well as clinically[9]. If comparable synergistic activities were manifest for piperacillin and the aminoglycosides, then currently used high carbenicillin/ticarcillin dosages with associated complications could be decreased by the lower doses of piperacillin. Similarly reduced doses of potentially toxic aminoglycosides could be used.

This investigation used techniques previously described[2,10] to study the *in vitro* efficacy of piperacillin, carbenicillin, and gentamicin. In addition, a large number of clinical bacterial isolates, including aminoglycoside-resistant bacteria were tested by 3 methods of synergy analysis.

Materials and Methods

Antimicrobics

Piperacillin was provided by Lederle Laboratories, Pearl River, New York; carbenicillin was a gift from Beecham Laboratories, Bristol, Tennessee; and gentamicin standard powders were generously provided by Schering Corporation, Bloomfield, New Jersey.

Organisms

A total of 405 bacterial strains were contributed by 3 collaborating laboratories, plus others kindly provided by Drs. T. L. GAVAN, Cleveland Clinic Foundation, Cleveland, Ohio; E. HUGH GERLACH, St. Francis Hospital, Wichita, Kansas; and P. C. FUCHS, St. Vincent Hospital, Portland, Oregon. These included 182 strains of Enterobacteriaceae, 153 strains of non-enterobacteriaceae Gram-negative bacilli, and 70 isolates of Gram-positive cocci. They were further classified by the following genus/species groups: *Escherichia coli* (24), *Klebsiella pneumoniae* (25), *Enterobacter* species (25), *Proteus mirabilis* (25), *Proteus* species, indol-positive (30), *Serratia marcescens* (28), *Providencia* species (25), *Pseudomonas aeruginosa* (100), *Pseudomonas* species (30), *Acinetobacter anitratus* (15), *Aeromonas hydrophila* (8), *Staphylococcus aureus* – penicillinase-positive (26), *Staphylococcus aureus* – penicillinase-negative (24), *Staphylococcus aureus* methicillin-resistant (10), and *Streptococcus faecalis* (10). These isolates were different from those previously reported[2].

Antimicrobic Susceptibility Testing

Minimum inhibitory concentrations (MIC) were determined by broth microdilution techniques. MUELLER-HINTON broth supplemented with calcium (50 mg/liter) and magnesium (25 mg/liter) were commercially dispensed in a single lot of plastic trays (Prepared Media Laboratory, Portland, Oregon) and distributed to the 3 testing laboratories. The trays were stored at $-60\sim-70$°C until used. Prior to inoculation the trays were thawed to room temperature and inoculum transferred to the wells by disposable plastic inoculators (Micro-Media Systems, San Jose, California) said to deliver 5 μl/well. Inoculum concentration was 5×10^5 CFU/ml. The MIC was recorded as the lowest antimicrobial concentration totally inhibiting organism proliferation (clear well) after $15\sim18$ hours of incubation at 35°C in a forced air incubator.

Antimicrobial Synergy Testing

One hundred and forty-two organisms were tested for synergy using gentamicin in combination with either piperacillin or reference carbenicillin. Tests were performed using checkerboard isobologram in microdilution broth trays, MIC decreases (4-fold) using MIC/4 concentrations of gentamicin and kill curves using MIC/4 concentrations of gentamicin, carbenicillin, and piperacillin alone and in combination.

The criteria for synergy[4] were as follows: (a) graphic concavity of the isobologram results; (b) 4-fold or more MIC decrease of both tested antimicrobics (gentamicin and either piperacillin or carbenicillin) when combined with the MIC/4 concentration of the others, and (c) a difference of 1 or more log kill favoring the combination over the best single antimicrobic at 4-hour incubation. Partial synergy was defined as less than or equal to 4-fold decrease in the MIC of one antimicrobic and a 2-fold reduction in the MIC of the other agent. The definition of indifference was no significant MIC reduction of either compound or only a 2-fold decrease of one compound. Antagonism was defined as a 4-fold or greater increase in the MIC values of either antimicrobic.

Results

The modal and range MIC values for piperacillin, carbenicillin and gentamicin against Gram-negative organisms are presented in Table 1. The 100 *Pseudomonas aeruginosa* strains are dominated by aminoglycoside-resistant isolates. Piperacillin was most active being 16-fold more inhibitory than carbenicillin and twice as active as gentamicin. Most of the 30 *Pseudomonas* species strains (5 species including *Pseudomonas acidovorans, Pseudomonas cepacia, Pseudomonas fluorescens-putida, Pseudomonas maltophilia* and *Pseudomonas stutzeri*) were inhibited by clinically achievable levels of piperacillin. Gentamicin was most active against *Acinetobacter* and *Aeromonas* strains; however, these species were also sensitive to the penicillins tested. Against the Enterobacteriaceae piperacillin was $4\sim>16$-fold more active than carbenicillin. Gentamicin was superior to either penicillin for all enterics except *Proteus – Providencia*. Piperacillin was most active against *Proteus* and carbenicillin against

Table 1. Modal and range MIC values for 335 Gram-negative bacilli from 9 genera (10 organism groups).

Organism (No.)	Modal (Range) MICs in μg/ml for:		
	Piperacillin	Carbenicillin	Gentamicin
Pseudomonas aeruginosa (100)	8 (1～>256)	128 (2～>256)	16 (0.25～>64)
Pseudomonas species[a] (30)	8 (≤0.5～>256)	>256 (≤0.5～>256)	>256 (0.25～>256)
Acinetobacter anitratus (15)	16 (8～64)	32 (8～64)	1 (1～>256)
Aeromonas hydrophila (8)	4 (≤0.5～16)	64 (8～>256)	1 (0.5～2)
Escherichia coli (24)	2 (1～>256)	8 (4～>256)	1 (0.5～2)
Klebsiella pneumoniae (25)	16 (4～>256)	>256 (128～>256)	1 (0.25～16)
Enterobacter species[b] (25)	4 (1～>256)	32 (0.5～>256)	1 (0.25～4)
Serratia marcescens (28)	4 (2～>256)	64 (2～>256)	1 (1～32)
Proteus mirabilis (25)	≤0.5 (≤0.5～2)	2 (≤0.5～16)	1 (0.5～4)
Proteus species[c] (30)	1 (≤0.5～128)	4 (1～>256)	1 (0.5～16)
Providencia species (25)	8 (≤0.5～>256)	4 (≤0.5～>256)	16 (0.5～>64)

[a] Includes *Pseudomonas cepacia* (3), *Ps. maltophilia* (3), *Ps. acidovorans* (3), *Ps. fluorescens-putida* (11) and *Ps. stutzeri* (10).
[b] Includes *Enterobacter cloacae* (10), *E. aerogenes* (10) and *E. agglomerans* (5).
[c] Includes all indole positive species, *Proteus morganii* (10), *Proteus rettgeri* (10) and *Proteus vulgaris* (10).

Table 2. Modal and range MIC results for 70 Gram-positive cocci including methicillin and penicillin resistant strains.

Organism (No.)	Modal (Range) MICs in μg/ml for:		
	Piperacillin	Carbenicillin	Gentamicin
Staphylococcus aureus, penicillinase-positive (26)	16 (4～>256)	16 (8～>256)	0.5 (≤0.125～32)
Staphylococcus aureus, penicillinase-negative (24)	1 (0.5～2)	1 (0.5～2)	0.5 (≤0.125～32)
Staphylococcus aureus, methicillin-resistant (10)	>256 (128～>256)	128 (64～256)	0.5 (≤0.125～1)
Streptococcus faecalis (10)	4 (4～8)	64 (32～128)	32 (8～64)

the *Providencia*. Nearly all *Providencia* species were resistant to gentamicin.

Table 2 shows the modal and range MIC values for 4 Gram-positive cocci groups. Staphylococci producing penicillinase have modal piperacillin and carbenicillin MIC values 16-fold higher than the penicillinase-deficient staphylococci. The MIC ranges are distinct and do not overlap. The piperacillin and carbenicillin MIC value below which one can predict sensitivity (non-penicillinase producer) appears to be less than or equal to 2 μg/ml. The methicillin-resistant *S. aureus* strains had much higher penicillin MICs, but all were sensitive to gentamicin. Only piperacillin demonstrated significant activity against *Streptococcus faecalis* confirming previous reports[1~5].

Fig. 1 demonstrates the kill curves of 5 representative species of bacteria. These show definite synergistic activity with greater than 2 and 4 log decrease in the viable cell counts at 4 and 24 hours respectively.

Fig. 2 demonstrates the isobologram plots of 2 representative *Pseudomonas aeruginosa* strains (012298 and 036932) showing synergistic interaction of piperacillin and gentamicin. One isolate was resistant (16 μg/ml) to gentamicin and the other sensitive. Both were sensitive to piperacillin at

Fig. 1. Kill curves demonstrating the bacteriocidal activity of piperacillin and gentamicin against 5 bacterial strains (*Enterobacter aerogenes* 035693, *Klebsiella pneumoniae* 036942, *Proteus morganii* 023229, *Pseudomonas aeruginosa* 012298 and *Serratia marcescens* 036951).

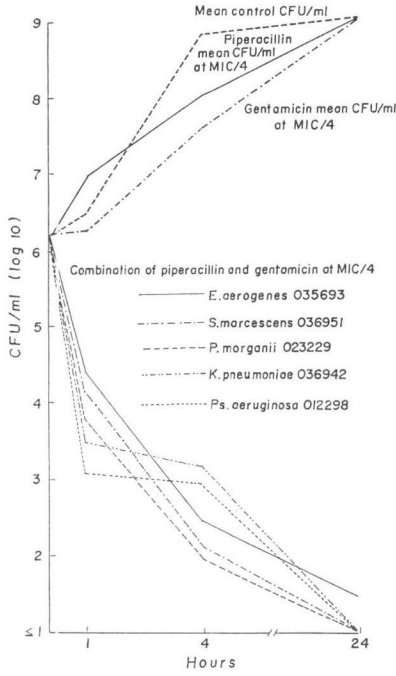


Fig. 2. Isobologram of 2 *Pseudomonas aeruginosa* strains demonstrating synergy to the combination of piperacillin and gentamicin. Isolate 012298 was resistant to gentamicin.

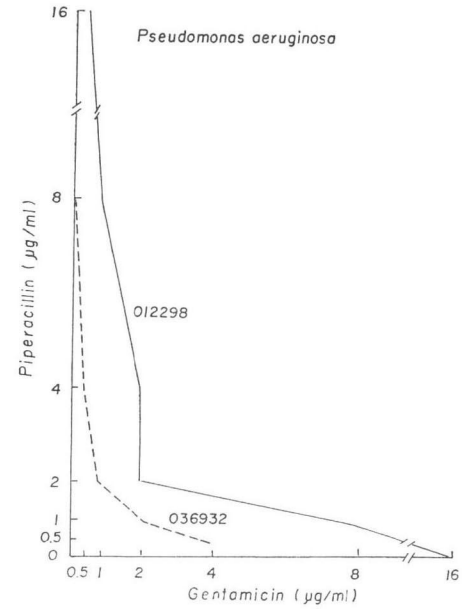
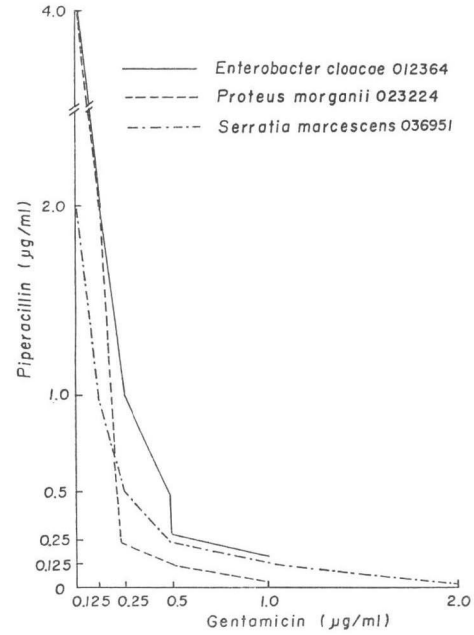


Fig. 3. Isobologram of 3 *Enterobacteriaceae* showing synergistic effects of piperacillin and gentamicin.



clinically achievable concentrations. Similarly, Fig. 3 shows synergistic isobolograms for 3 piperacillin and gentamicin sensitive Enterobacteriaceae.

Table 3 reports the overall synergy, partial synergy, and indifferent results indexed by species group, antibiotic combinant and gentamicin susceptibility. In all cases comparative piperacillin and carbenicillin data were available with the exception of *Klebsiella pneumoniae* which were all carbenicillin-resistant *e.g.* MIC > 256 μg/ml. Of the 142 strains tested for synergy, 52 were non-Enterobacteriaceae Gram-negative bacilli of which 65% were gentamicin-resistant *Pseudomonas aeruginosa* (MICs equal to or greater than 16 μg/ml). The occurrence of synergy among this subgroup was somewhat variable. Gentamicin in combination with either penicillin was synergistic or partially synergistic against 97% of the *Pseudomonas aeruginosa* strains.

Table 3. The results of antibiotic interaction studies of gentamicin combined with piperacillin or carbenicillin for 142 clinical bacterial isolates.

Organism (No.)	Antibiotic	Gentamicin susceptibility[a]	Antibiotic interaction No. (%)		
			Synergy	Partial synergy	Indifferent
Pseudomonas aeruginosa (34)	Piper	S	4 (80)	1 (20)	0 (0)
		R	25 (86)	3 (11)	1 (3)
	Carb	S	1 (20)	3 (60)	1 (20)
		R	27 (93)	2 (7)	0 (0)
Pseudomonas species (5)	Piper	S	1 (20)	2 (40)	2 (40)
	Carb	S	1 (20)	2 (40)	2 (40)
Acinetobacter anitratus (8)	Piper	S	0 (0)	3 (38)	5 (62)
	Carb	S	0 (0)	3 (38)	5 (62)
Aeromonas hydrophila (5)	Piper	S	1 (20)	2 (40)	2 (40)
	Carb	S	1 (20)	2 (40)	2 (40)
Escherichia coli (13)	Piper	S	1 (7)	3 (24)	9 (69)
	Carb	S	0 (0)	0 (0)	13 (100)
Klebsiella pneumoniae (10)	Piper	S	2 (22)	6 (67)	1 (11)
		R	1 (100)	0 (0)	0 (0)
Enterobacter species (15)	Piper	S	2 (13)	5 (33)	8 (54)
	Carb	S	1 (7)	9 (60)	5 (33)
Serratia marcescens (16)	Piper	S	2 (13)	9 (56)	5 (32)
	Carb	S	2 (13)	7 (44)	7 (44)
Proteus species (15)	Piper	S	3 (23)	2 (16)	8 (61)
		R	1 (50)	1 (50)	0 (0)
	Carb	S	2 (16)	6 (46)	5 (38)
		R	1 (50)	0 (0)	1 (50)
Providencia stuarti (12)	Piper	S	2 (100)	0 (0)	0 (0)
		R	10 (100)	0 (0)	0 (0)
	Carb	S	1 (100)	0 (0)	1 (50)
		R	7 (70)	2 (20)	1 (10)
Staphylococcus aureus (6)	Piper	S	1 (17)	2 (33)	3 (50)
	Carb	S	0 (0)	1 (17)	5 (83)
Streptococcus faecalis (3)	Piper	R	0 (0)	1 (33)	2 (67)
	Carb	R	0 (0)	2 (67)	1 (33)

[a] S=susceptable (MIC≤4 μg/ml) or R=resistant (MIC≥16 μg/ml).

Table 4. Antimicrobial interaction summary for gentamicin in combination with piperacillin and carbenicillin. Eleven bacterial genera were tabulated including 45 gentamicin resistant (MIC=16 μg/ml) strains.

Antibiotic	Gentamicin susceptibility (No.)	Antibiotic interaction in %		
		Synergy	Partial synergy	Indifferent
Piperacillin	Sensitive (97)	19	36	45
	Resistant (45)	82	11	7
	Total (142)	38	28	34
Carbenicillin	Sensitive (88)	11	37	52
	Resistant (44)	79	13	8
	Total (132)	34	29	37

However, only a single strain each of the *Pseudomonas stutzeri* and *Aeromonas hydrophila* showed synergistic activity.

Among the remaining Enterobacteriaceae and Gram-positive cocci, the combination of piperacillin-gentamicin was superior to carbenicillin-gentamicin against *E. coli, Staphylococcus aureus* and

Klebsiella. Both drug combinations were equally active against *Serratia marcescens, Proteus – Providencia* group, and *S. faecalis*. The carbenicillin-gentamicin combination proved to be superior only against isolates of *Enterobacter cloacae* and *Enterobacter aerogenes*.

Table 4 summarizes the synergy results for the 142 strains. No statistical advantage could be identified favoring either antimicrobial combination. Piperacillin and carbenicillin combinations showed synergistic or partially synergistic activity against 66% and 63% of the strains, respectively. However, the synergy incidence of both combination versus the gentamicin-resistant strains was approximately 4-fold more frequent than in the sensitive population. Antagonism was not found in this study.

Discussion

The *in vitro* comparison of piperacillin and carbenicillin was similar to those described previously[1~5]. Though piperacillin MIC results were generally higher by species group than we previously reported[2,5], the organism population in this study of more resistant bacteria maintained the same proportional activity advantage of piperacillin over carbenicillin. Gentamicin was more active than piperacillin against nearly all bacterial species tested except *Pseudomonas aeruginosa, Pseudomonas* species, *Providencia stuarti* and *S. faecalis*. Carbenicillin was the superior agent only against gentamicin-resistant *Providencia stuarti* isolates. Both penicillins were relatively inactive against penicillin-resistant *S. aureus*; they seem to be very susceptible to staphylococcal beta-lactamase. Definite bimodal population distributions are noted with the beta-lactamase-producing strains MICs ≥ 4 μg/ml. A separate zone size for piperacillin and carbenicillin may be advised for staphylococci similar to those used for penicillin G and ampicillin[11].

Gentamicin-resistant *Pseudomonas aeruginosa* generally seems to possess higher resistances to anti-pseudomonas semisynthetic penicillins than do aminoglycoside-sensitive isolates. This observation was cited earlier[2] and confirmed with these data. All previous reports of median *Pseudomonas aeruginosa* piperacillin MIC values range between 2 and 4 μg/ml[1~5] except for the aminoglycoside-resistant populations.

Although piperacillin alone against aminoglycoside-resistant *Pseudomonas aeruginosa* may be effective, antimicrobial interaction studies demonstrate a high synergistic rate using piperacillin and gentamicin in combination. Fu and Neu found that 67% of these organisms would be either synergistic or partially synergistic[4]. We found that 84% were synergistic and 13% partially synergistic to a combination of piperacillin and gentamicin. Carbenicillin plus gentamicin was equally efficacious *in vitro*. Aminoglycoside-resistant *Providencia* was found more likely to be synergistic than those strains previously reported[4]. Also, partial synergy was identified for over half of our *Serratia marcescens* group in contrast to a dominant synergistic population found by Fu and Neu. Our findings of 27% synergy between piperacillin and gentamicin against *K. pneumoniae* contrasts to the non-contributory effects of carbenicillin combined with gentamicin against the same population of organisms. Against all other organisms the combinations were usually indifferent or partially synergistic. These include *Aeromonas, Enterobacter,* indol-positive *Proteus* species, *S. aureus, A. anitratus, Citrobacter*[4], *Salmonella*[4], and *Shigella*[4]. The overall incidence of synergy and partial synergy for piperacillin-gentamicin found by Fu and Neu was 60%. We documented a similar rate of 66% and a carbenicillin-gentamicin rate of 61%.

The *in vitro* activity and synergy comparisons demonstrate piperacillin to be a highly promising beta-lactam antimicrobic as previously described. *In vivo* animal and clinical studies using piperacillin alone and in combination with aminoglycosides should be encouraged particularly for Gram-positive and Gram-negative endocarditis, Gram-negative bacteremias, and *Klebsiella pneumoniae* pneumonia.

References

1) Ueo, K.; Y. Fukuoka, T. Hayashi, T. Yasuda, H. Taki, M. Tai, Y. Watanabe, I. Saikawa & S.

MITSUHASHI: *In vitro* and *in vivo* antibacterial activity of T-1220, a new semisynthetic penicillin. Antimicr. Agents & Chemoth. 12: 455~460, 1977

2) JONES, R. N.; C. THORNSBERRY, A. L. BARRY, P. C. FUCHS, T. L. GAVAN & E. H. GERHLACH: Piperacillin (T-1220), a new semisynthetic penicillin. I. *In vitro* antimicrobial activity comparison with carbenicillin, ticarcillin, ampicillin, cephalothin, cefamandole and cefoxitin. J. Antibiotics 30: 1107~1114, 1977
3) VERBIST, L.: *In vitro* activity of piperacillin, a new semisynthetic penicillin with an unusually broad spectrum of activity. Antimicr. Agents & Chemoth. 13: 349~357, 1978
4) FU, K. P. & H. C. NEU: Piperacillin, a new penicillin active against many bacteria resistant to other penicillins. Antimicr. Agents & Chemoth. 13: 359~367, 1978
5) GERLACH, E. H.; P. C. FUCHS, R. N. JONES, T. L. GAVAN, A. L. BARRY & C. THORNSBERRY: Piperacillin: A collaborative *in vitro* comparison with carbenicillin against 10,838 clinical bacterial isolates. Curr. Micro. (In Press) 1978
6) LIBKE, R. D.; C. RAGAMEY, J. T. CLARK & W. M. M. KIRBY: Synergism of carbenicillin and gentamicin against enterococci. Antimicr. Agents & Chemoth. 4: 564~568, 1973
7) KLUGE, R. M.; H. C. STANDIFORD, B. TATEM, V. M. YOUNG, W. H. GREENE, S. C. SCHIMPFF, F. M. CALIA & R. B. HORNICK: Comparative activity of tobramycin, amikacin and gentamicin alone and with carbenicillin against *Pseudomonas aeruginosa*. Antimicr. Agents & Chemoth. 6: 442~446, 1974
8) FU, K. P. & H. C. NEU: Amikacin *in vitro* activity against multiresistant bacteria used singly and in combination with penicillins. U.S. Amikacin Symposium, Am. J. Med. (Suppl.), June, 1977
9) KLASTERSKY, J.; R. CAPPEL & D. DANEAU: Clinical significance of in vitro synergy between antibiotics in gram-negative infections. Antimicr. Agents & Chemoth. 2: 470~475, 1972
10) JONES, R. N.; C. THORNSBERRY, A. L. BARRY, P. C. FUCHS, T. L. GAVAN & E. H. GERLACH: BL-S786, a new parenteral cephalosporin. II. *In vitro* antimicrobial activity comparison with six related cephalosporins. J. Antibiotics 30: 583~592, 1977
11) BAUER, A. W.; W. M. M. KIRBY, J. C. SHERRIS & M. TURCK: Antibiotic susceptibility testing by a standardized single disc method. Am. J. Clin. Path. 45: 493~496, 1966