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Synergy of Mecillinam (FL1060) with Penicillins and Cephalosporins Against *Proteus* and *Klebsiella*, with Observations on Combinations with Other Antibiotics and Against Other Bacterial Species

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Thirty-five strains each of *Klebsiella* and *Proteus* were tested for susceptibility to mecillinam alone and in combination with ampicillin, carbenicillin, cephalothin, and cefazolin. Antibiotics were considered to be synergistic when there was a ≥fourfold reduction in minimum inhibiting concentration of both antibiotics in the combination as compared with that of each antibiotic alone. Synergy of mecillinam with ampicillin, carbenicillin, cephalothin, and cefazolin was demonstrated against 2, 3, 7, and 8 of the 35 strains of *Klebsiella* and against 14, 14, 19, and 24 of the 35 strains of *Proteus*, respectively. Synergy against the isolates of *Proteus* was related to species, whereas against *Klebsiella* it was related to susceptibility of the isolates to mecillinam. Tests of combinations of mecillinam with other antibiotics on the same and different species indicated that synergy was related to the antibiotic, the species, and the strains of organisms tested.

Mecillinam (MCN) is one of a new class of semisynthetic penicillins, the 6-β-amidinopenicillanic acids (Fig. 1). (MCN and pivmecillinam are used in Europe as the generic designates of FL-1060 and FL-1039, respectively, but as of the date of this writing they are not adopted as generic designations in the U. S.) Like other active penicillins, it is a substituted 6-aminopenicillanic acid. Little, if any, MCN is absorbed after oral administration. However, the pivaloyloxy-methyl ester (FL1039) is well absorbed when given by mouth; it is rapidly converted to active MCN in the blood and is excreted as MCN in the urine.

The first report in 1972 by Lund and Tybring (5) of Leo Pharmaceutical Products Ltd. indicated that MCN has bactericidal activity against a variety of gram-negative rods but little or none against *Pseudomonas, Haemophilus influenzae,* and gram-positive cocci. This unusual spectrum suggested a mode of action different from that of other penicillins. Morphological studies of antibiotic-damaged bacteria by Lund and Tybring (5), amplified by those of Greenwood and O'Grady (4) and Melchior et al. (7), supported this suggestion. Exposed rods were converted to spherical forms different from those of penicillin-damaged rods.

In 1973, Park and Burman (8) showed that, in addition to differing from other penicillins in its effect on the morphology of the organisms, the enzymatic blocks of cell wall synthesis by MCN were different, especially the absence of inhibition of murein transpeptidase.

The possibility of a penicillin with solely gram-negative activity and bactericidal activity at a different site suggested that MCN might have activity which is additive or synergistic with other β-lactam antibiotics, the one causing sublethal inhibition of cell wall synthesis at one site and the other producing lethal damage by inhibition at another site, in organisms that are only moderately susceptible to inhibition by the agents separately. More recently, Tybring and Melchior (12) reported synergy of MCN in bactericidal action against 14 strains of *Enterobacteriaceae* including strains of *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris,* and *Salmonella typhi*. In this paper we report the results of tests for synergy using 35 strains each of two species of gram-negative bacilli which differ in their susceptibility to MCN and to other β-lactam antibiotics.

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MATERIALS AND METHODS

Bacteria. The strains of *Proteus* and *K. pneumoniae* used in this study were isolated from the blood

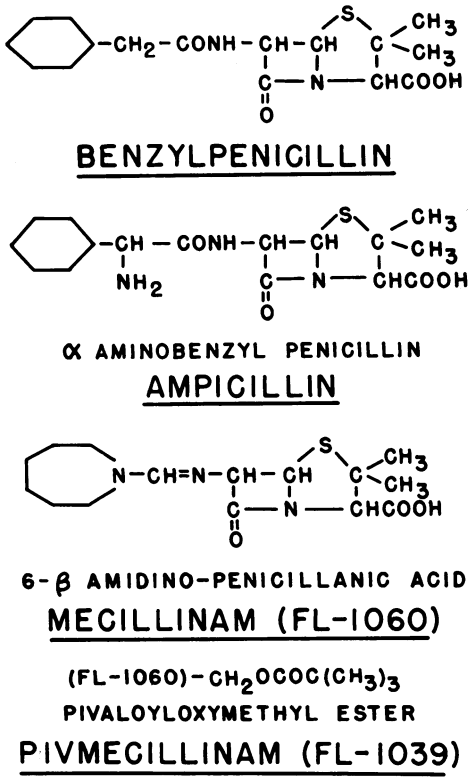

FIG. 1. *Structures of MCN and pivmecillinam as compared with benzylpenicillin and AMP. The ester in FL-1039 substitutes for the terminal H in FL-1060.*

of bacteremic patients hospitalized during 1972 at Boston City Hospital and were from among those employed in an earlier study reported by McGowan et al. (6).

Susceptibility tests. These were performed by the agar-dilution method employing the inocula-replicator of Steers, Foltz, and Graves (10). Overnight cultures in heart infusion broth were diluted 10^{-3} and applied to antibiotic-containing plates made with NIH broth with 1.5% agar (Difco) added. The first batch of NIH broth used had a pH of 6.82 at 22 C, conductivity of 7.5 $mohm^{-1}/cm$ at 22 C and osmolality of 212 mosM/kg. NIH broth (Difco) and NIH agar (Difco) were used in later studies, both pH 7.1 (11, 12). The minimum concentration (MIC) was noted as the lowest concentration of antibiotic allowing no visible growth or ≤5 small colonies after incubation for 20 to 24 h at 37 C. When two antibiotics were used together they were considered to be synergistic when there was a fourfold or greater reduction of MIC of both agents as compared with that of each antibiotic alone. Tests for synergy were done with antibiotics combined in equal concentrations and also in a checkerboard pattern to provide isobolograms.

Antibiotics. Mecillinam, as dry crystals, was supplied by Emanuel Grunberg, Hoffmann-LaRoche, Inc. Assay standards of other antibiotics were provided as dry powders; they were diluted in water on the day of each test according to the labeled potency of each standard.

RESULTS

Antibacterial activity. Figure 2 shows the MICs of 35 strains of *Proteus* species to MCN and the four other antibiotics used in tests for synergy. Only six (17%) of the 35 strains of *Proteus* species were inhibited by 6.25 μg or less of MCN/ml. The 35 strains included 11 of *P. mirabilis*, nine of *P. vulgaris*, and three of *P. rettgeri*. The strains of *P. mirabilis* and *P. vulgaris* showed patterns of MICs similar to that of the combined group. However, the 12 strains of *P. morganii* were all resistant to 100 μg of MCN/ml. MCN was the least active of the five antibiotics, carbenicillin (CARB) was the most active, and the activity of cefazolin (CZ), cephalothin (CTN), and ampicillin (AMP) was intermediate.

Figure 3 shows that the 35 isolates of *K. pneumoniae* that were tested were much more susceptible to MCN than were those of *Proteus*: 28 (80%) of them were inhibited by ≤6.25 μg/ml. CZ, CTN, AMP, and CARB were all considerably less active than MCN against most of the strains, and the order of their activity was the reverse of that against *Proteus*.

Tests for synergy with equal concentrations of antibiotics. Table 1 shows the results of tests for synergy with combinations of MCN with four β-lactam antibiotics against 35 strains of *Proteus* and 35 of *Klebsiella* using equal concentrations of each agent. Against *Proteus* the combinations with CZ and CTN were synergistic for 69 and 54%, respectively, whereas each of the combinations of MCN with AMP and CARB was synergistic against 40% of the strains.

P. mirabilis is generally more susceptible to

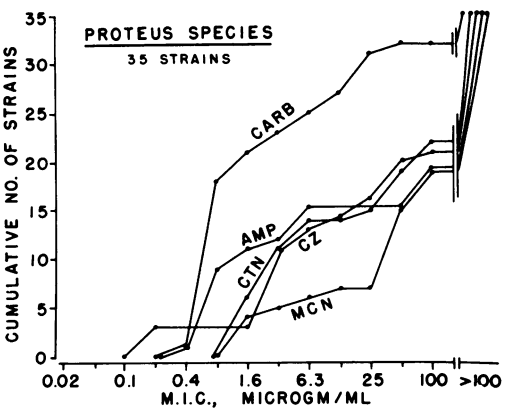

FIG. 2. *Susceptibility of 35 strains of Proteus to MCN, CARB, AMP, CTN, and CZ.*

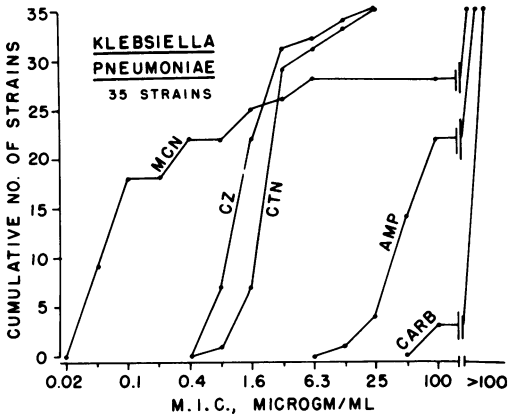

FIG. 3. *Susceptibility of 35 strains of K. pneumoniae to MCN CZ, CTN, AMP, and CARB.*

TABLE 1. *Synergy of mecillinam with other β-lactam antibiotics*[a]

Organism	No. tested	No. showing synergy of MCN plus:			
		AMP	CARB	CTN	CZ
Proteus rettgeri	3	0	0	1	1
P. morganii	12	0	1	0	6
P. vulgaris	9	3	3	7	6
P. mirabilis	11	11	10	11	11
All *Proteus*	35	14	14	19	24
Klebsiella pneumoniae	35	2	3	7	8

[a] Synergy is defined as ≥fourfold reduction in MIC for each when combined in ratio 1:1.

penicillins and cephalosporins than the indole-positive species of *Proteus*. To some extent this was shown also for synergy with MCN (Table 1). All 11 strains of *P. mirabilis* were inhibited synergistically with all of the combinations except that with CARB, which was synergistic with MCN against all but one of the 11 strains. Among the indole-positive species, all 12 strains of *P. morganii* were highly resistant to MCN alone; the combination with CZ was synergistic for one-half of the strains, and with CTN against only one of the 12 strains, whereas the combinations of MCN with AMP and CARB were not synergistic against any of the strains of *P. morganii* tested. *P. vulgaris* differed from *P. morganii* in that all four of the β-lactam antibiotics were synergistic with MCN against some strains; three of the nine were inhibited synergistically by MCN and the two penicillins, and seven and six of them by the combination of MCN with CTN and CZ, respectively. There were only three strains of *P. rettgeri;* synergy was demonstrated with one of them in the combinations with each of the cephalosporins.

Strains of *K. pneumoniae* were inhibited less frequently than strains of *Proteus* with the same combinations and, as with *Proteus*, there were more strains inhibited synergistically with the cephalosporins than with the penicillins. Synergy against strains of *Proteus* was related to the species; however, with *Klebsiella* the frequency of synergy of MCN with other β-lactam antibiotics correlated only with the susceptibility to MCN alone, as shown in Table 2. More than two-thirds of the strains of *Klebsiella* were inhibited by MCN alone at 0.8 μg/ml but only one of these sensitive strains was inhibited synergistically, and with only one of the β-lactam antibiotics, namely CTN. On the other hand, strains with MIC ≥ 1.6 μg/ml were more susceptible to the combination of antibiotics, and again CZ was the most active, showing synergy with eight of these nine strains.

Combinations of MCN with other antibiotics. Synergy against strains of *P. mirabilis* was also noted with MCN combined with other antibiotics including benzylpenicillin, penicillinase-resistant penicillins, and other cephalosporins. Of the aminoglycoside antibiotics, only amikacin was synergistic with as many as 10 of 11 strains, whereas gentamicin and tobramycin were each synergistic with MCN against 4 of 11 strains. Moreover, the aminoglycosides were synergistic with MCN only against *P. mirabilis* but not against strains of other species of *Proteus*.

In screening many other antibiotics in combination with MCN against the 12 strains of *P. morganii* only the combination with chloramphenicol and clindamycin acted synergistically against as many as 7 of the 12 strains.

Isobolograms. Synergy of MCN with β-lac-

TABLE 2. *Correlation of susceptibility of K. pneumoniae to mecillinam and occurrence of synergy with other β-lactam antibiotics*

MCN plus:	No. of strains showing synergy		
	Total	MIC of MCN (μg/ml)	
		≥1.6	≤0.8
AMP	2	2	0
CARB	3	3	0
CTN	7	6	1[a]
CZ	8	8	0
No. tested	35	9	26

[a] Uncertain end point (incomplete inhibition).

tam antibiotics was also demonstrated by using combinations in checkerboard dilutions from which the results could be displayed as isobolograms; the points on the curve where both antibiotics are less than one-fourth of the MIC of each antibiotic alone are demonstrations of synergy. Figure 4 shows the isobologram obtained with MCN and CZ against *P. vulgaris* 33, and Fig. 5 shows a similar isobologram for the same combination against *K. pneumoniae* 29. The combination was highly synergistic against both strains.

Antibacterial activity of MCN against strains of other species. Isolates of several other species were tested against MCN with results similar to those reported by Lund and Tybring (5).

Among seven strains of *E. coli* resistant to 100 μg of AMP/ml, six were inhibited by ≤0.8 μg of MCN/ml. Four strains of *Pseudomonas aeruginosa* were resistant to 100 μg of all five of the β-lactam antibiotics tested per ml, including MCN. All of 35 strains of enterococci were highly resistant to MCN alone, and no synergy was demonstrable when MCN was combined with benzylpenicillin, AMP, CTN, or gentamicin in tests using checkerboard combinations of dilutions.

DISCUSSION

The data presented demonstrate a considerable degree of synergy of MCN with penicillin and cephalosporin antibiotics against some gram-negative rods. This lends support to the evidence suggesting that MCN has a site of action different from that of other β-lactam antibiotics. Thus, the 6-β-amidinopenicillanic acids represent a unique class of substituted 6-aminopenicillanic acids different from the natural penicillins, the semisynthetic penicillinase-resistant penicillins, and the AMP-like and CARB-like "broad-spectrum" penicillins.

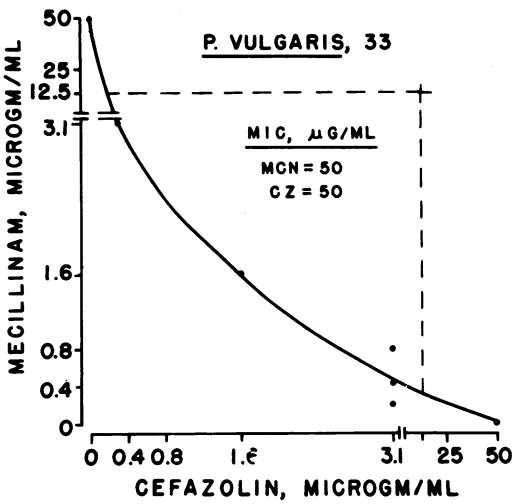

FIG. 4. *Isobologram showing synergistic action of MCN and CZ against P. vulgaris 33. The broken horizontal and vertical lines indicate 1/4 MIC of MCN and CZ, respectively.*

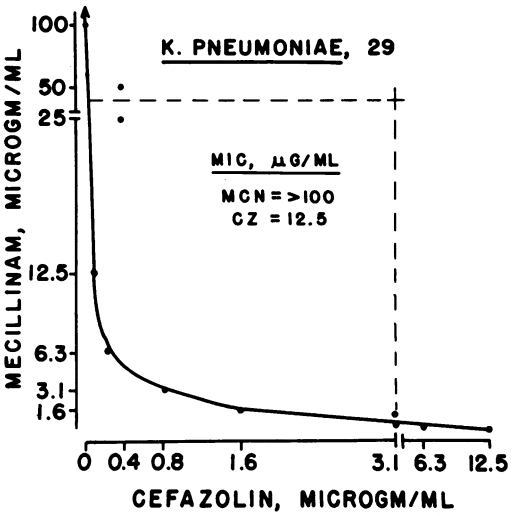

FIG. 5. *Isobologram showing synergy of MCN and CZ against K. pneumoniae 29. The broken horizontal and vertical lines indicate 1/4 MIC of MCN and CZ, respectively.*

The recognition, early in the development of this agent, of its activity in synergistic combinations is similar to the development of the combination of trimethoprim with sulfamethoxazole. Trimethoprim/sulfamethoxazole was notable as the first antimicrobial agent introduced as a fixed-dose combination for systemic use which was based on the evidence for blocking at two successive stages of the bacterial biosynthetic pathway (for folic acid synthesis), which was borne out in vitro and also in studies of bacterial infections in vivo (2).

In contrast to the trimethoprim/sulfamethoxazole combination, which was shown to act synergistically against the great majority of strains of susceptible species (1), the synergistic action of MCN with other antibiotics is much more selective, not only with regard to the other antibiotics in the combination and the species of the organism, but also the individual strains within each species. Similar strain specificity was demonstrated previously in this laboratory with respect to the synergistic action of the combinations of CARB with gentamicin, polymyxin, and kanamycin against strains of *P. aeruginosa* (9) and with the combination of

erythromycin and AMP against strains of *Nocardia asteroides* (3). Synergy by the latter combination was related to the susceptibility of the strain to erythromycin, just as was shown in the present study for synergy of MCN with penicillin and cephalosporin antibiotics against strains of *Klebsiella*.

Although this report deals with the activity of MCN against *Proteus* and *Klebsiella*, there is evidence of its synergistic activity against other *Enterobacteriaceae* (11), and evidence has been presented that MCN acts in synergy with penicillins and cephalosporins in an in vivo mouse model (4a).

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