ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1982, p. 518–519
0066-4804/82/090518-02$02.00/0

Vol. 22, No. 3

Synergistic Activity of Mecillinam in Combination with the β-Lactamase Inhibitors Clavulanic Acid and Sulbactam

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Received 10 March 1982/Accepted 24 June 1982

The β-lactamase inhibitors clavulanic acid and sulbactam were combined with mecillinam. β-Lactamase-containing *Escherichia coli* resistant to mecillinam was synergistically inhibited by both clavulanic acid and sulbactam. β-Lactamase-containing *Enterobacter* was synergistically inhibited, but strains lacking β-lactamases were not synergistically inhibited. Synergistic inhibition was noted for β-lactamase-containing, mecillinam-resistant *Klebsiella, Citrobacter, Serratia,* and *Salmonella* isolates, but only 18% of β-lactamase-containing *Proteus mirabilis, Providencia rettgeri, Providencia stuartii,* and *Morganella morganii* were synergistically inhibited by the combinations.

Mecillinam is an amidino penicillin which inhibits a large number of gram-negative bacilli resistant to ampicillin, even though it is hydrolyzed by many of the common plasmid and chromosomal β-lactamases (5, 8). In earlier studies, we had shown that mecillinam resistance in some organisms could be correlated with production of β-lactamases. For this reason, we evaluated the effect of the combination of mecillinam with clavulanic acid and sulbactam, compounds which have been shown to be effective β-lactamase inhibitors (1–4, 6).

All organisms were clinical isolates which had been saved because of the presence of β-lactamases detected by the nitrocefin method (7). Clavulanic acid was a gift of Beecham Laboratories, sulbactam was obtained from Pfizer Inc., and mecillinam was obtained from Roche Laboratories. Synergy of the compounds was determined by an agar dilution method with NIH agar (5) and an inoculum of 10^5 colony-forming units. Clavulanic acid and sulbactam were present at concentrations of 1 and 5 μg/ml. Synergy was defined as a fourfold or greater reduction in the minimal inhibitory concentration (MIC) of mecillinam. The clavulanic acid and sulbactam MICs for all isolates were ≥25 μg/ml. Organisms were considered to be resistant to mecillinam if the MICs were >12.5 μg/ml.

Clavulanic acid acted synergistically with mecillinam against all mecillinam-resistant isolates of *Escherichia coli*, but the combination showed synergy for only one-third of the susceptible *E. coli* isolates. The combination of mecillinam and clavulanic acid was also synergistic for 5 of 6 mecillinam-resistant *Klebsiella* isolates, but synergy was found only with 1 of 11 susceptible isolates. Synergy of clavulanic acid and mecillinam was demonstrated against mecillinam-resistant *Salmonella, Citrobacter,* and *Serratia,* but synergy was demonstrated against mecillinam-resistant isolates for only 2 of 4 *Enterobacter,* 3 of 12 *Providencia,* 3 of 5 *Providencia rettgeri,* 2 of 23 *Proteus mirabilis,* and 1 of 7 *Morganella morganii* isolates. Overall synergy was seen with 41% of 78 resistant isolates and with 19.5% of 41 mecillinam-susceptible isolates (Table 1).

The combination of mecillinam and sulbactam was synergistic for all of the *E. coli, Salmonella,* and *Citrobacter* isolates resistant to mecillinam but for only one of six mecillinam-resistant *Klebsiella* and two of four *Enterobacter* isolates. Synergy of mecillinam against *Proteus, Providencia,* and *Morganella* was infrequent. Overall, 32% of the mecillinam-resistant isolates were synergistically inhibited, and 26.8% of the susceptible isolates were synergistically inhibited by the combination of mecillinam and sulbactam (Table 1).

In general, clavulanic acid was more effective than sulbactam on a weight basis in lowering the mecillinam MIC values. The effect of increasing the concentration of sulbactam from 1 to 5 μg/ml caused a minimal improvement in synergy, whereas the increase of clavulanic acid from 1 to 5 μg/ml caused a major lowering of mecillinam MICs (Table 2).

Although mecillinam inhibits many β-lactamase-containing, gram-negative bacilli, some isolates are resistant (5). Mecillinam is hydrolyzed by some plasmid β-lactamases, albeit at a slower rate than is ampicillin (5, 8). This slower destruction of mecillinam by β-lactamases, com-

TABLE 1. Synergistic activities of combinations of mecillinam with clavulanic acid or sulbactam as determined by mecillinam resistance and susceptibility

Organism (no. of isolates)	No. isolates/total inhibited synergistically by mecillinam combined with[a]:			
	Clavulanic acid		Sulbactam	
	Me^r	Me^s	Me^r	Me^s
Escherichia coli (17)	5/5	4/12	5/5	0/12
Klebsiella pneumoniae (17)	5/6	1/11	1/6	1/11
Enterobacter cloacae (14)	1/3	2/11	1/3	10/11
Enterobacter agglomerans (1)	1/1	0/0	1/1	0/0
Providencia stuartii (15)	3/12	0/3	3/12	0/3
Providencia rettgeri (5)	3/5	0/0	0/5	0/0
Morganella (7)	1/7	0/0	0/7	0/0
Proteus mirabilis (23)	2/23	0/0	2/23	0/0
Salmonella typhimurium (2)	2/2	0/0	2/2	0/0
Serratia marcescens (8)	6/8	0/0	7/8	0/0
Citrobacter freundii (5)	2/2	1/3	2/2	0/3
Acinetobacter calcoaceticus (5)	1/4	0/1	1/4	0/1

[a] Me^r, Mecillinam resistant; Me^s, mecillinam susceptible.

TABLE 2. Effect of increased concentrations of clavulanic acid and sulbactam on mecillinam activity

Organism	MIC (μg/ml) of mecillinam				
	Alone	Plus clavulanic acid at:		Plus sulbactam at:	
		1 μg	5 μg	1 μg	5 μg
Escherichia coli	>50	0.4	0.5	3.1	3.1
Enterobacter cloacae	25	1.6	0.02	3.1	3.1
Citrobacter freundii	50	6.3	0.04	12.5	6.3
Serratia marcescens	>50	3.1	1.6	6.3	6.3
Klebsiella pneumoniae	>50	3.1	<0.1	25	12.5
Providencia rettgeri	>50	>50	>50	>50	1.6
Salmonella typhimurium	50	1.6	0.05	6.3	6.3

bined with a high affinity for penicillin-binding protein 2, has been suggested as the reason for the activity of mecillinam against *E. coli, Klebsiella*, and other *Enterobacteriaceae* resistant to ampicillin. Clavulanic acid and sulbactam both inhibit β-lactamases, primarily the plasmid enzymes and also the broad-spectrum *Klebsiella* enzyme (2–4, 6).

These studies demonstrate that these two β-lactamase inhibitors cause the majority of *E. coli, Klebsiella, Citrobacter, Salmonella*, and *Serratia* resistant to mecillinam to be inhibited by concentrations which could be achieved clinically. In contrast, neither β-lactamase inhibitor significantly increases the activity of mecillinam against *Proteus mirabilis, Providencia*, or *Acinetobacter*. Furthermore, neither clavulanic acid nor sulbactam increase the activity of mecillinam against a significant number of mecillinam-susceptible isolates. In general, clavulanic acid on a micrograms-per-milliliter basis was more effective at increasing the activity of mecillinam than was sulbactam.

These observations suggest that the combination of mecillinam with a β-lactamase inhibitor will extend the activity of this compound to inhibit many resistant isolates. Whether these in vitro observations can be utilized in human infections will be resolved only by further animal and human studies.

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