ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1990, p. 156–158
0066-4804/90/010156-03$02.00/0

Vol. 34, No. 1

Diverse Potential of β-Lactamase Inhibitors To Induce Class I Enzymes

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The ability of various β-lactamase inhibitors to induce class I β-lactamases was assessed. Clavulanate was the most active compound, inducing *Morganella morganii*, *Aeromonas caviae*, and *Enterobacter aerogenes* over a broad concentration range and *Citrobacter freundii*, *Pseudomonas aeruginosa*, and *Serratia marcescens* at high concentrations. Disk approximation tests paralleled these results, with clavulanate, but not sulbactam or tazobactam, antagonizing the activity of several β-lactams against these organisms.

It is now well established that particular β-lactam antibiotics (e.g., cefoxitin) are potent inducers of class I β-lactamases found in members of the family *Enterobacteriaceae* and in *Pseudomonas aeruginosa*. While such induction is reversible, occurring only during exposure to the inducing drug, it is sufficient to impart resistance to β-lactams in both in vitro and in vivo settings (9). The β-lactamase inhibitors (clavulanate, sulbactam, and tazobactam) are potentially important adjuvants to β-lactam chemotherapy. Each is a strong inhibitor of plasmid-mediated β-lactamases among gram-negative organisms, but the three drugs have little antibacterial activity and are not significant inhibitors of chromosomal β-lactamases (3, 7). However, these compounds as β-lactams represent new potential inducers of class I enzymes. Therefore, this study was designed to assess the ability of these β-lactamase inhibitors to induce chromosomal β-lactamases of *Aeromonas caviae*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Morganella morganii*, *P. aeruginosa*, and *Serratia marcescens*.

The inducer potencies of clavulanate, sulbactam, tazobactam, and their companion β-lactam antibiotics (ticarcillin, cefoperazone, and piperacillin, respectively) were quantitatively determined in induction assays with a single wild-type strain of each species. All strains used throughout this study were clinically isolated wild-type organisms susceptible to cefoperazone, piperacillin, and ticarcillin. Cefoxitin, a known potent inducer (11, 12), was included in all experiments for comparison. Powders of each study compound were obtained as follows: cefoxitin, Merck Sharp & Dohme, Rahway, N.J.; ticarcillin and clavulanate, Beecham Laboratories, Bristol, Tenn.; cefoperazone and sulbactam, Pfizer Pharmaceuticals, New York, N.Y.; and piperacillin and tazobactam, Lederle Laboratories, Pearl River, N.Y. Agar dilution MICs were determined in Mueller-Hinton medium by using an inoculum of 10^4 CFU per spot according to standard protocols (8). For each compound, inductions were performed above and below the MIC with concentrations of 0.1, 1.0, 10, and 100 μg/ml. Log-phase cells were exposed to the inducer for 2 h, and sonic extracts of both induced and uninduced cells were dialyzed overnight at room temperature in 0.1 M potassium phosphate buffer (pH 7.0) to remove excess inducer. Enzyme activity was determined in UV assays with 100 μM cephalothin as the substrate (11). All inductions were performed in duplicate, and the results represent the averages of the two determinations. β-Lactamase activity fivefold or more greater than uninduced activity was considered significant induction. Results are not given for those concentrations of inducer which produced significant killing under conditions present in the induction assays. Those compounds giving induction ratios (induced/uninduced enzyme activity) of <10 at all concentrations tested were considered weak inducers. Those with induction ratios of ≥ 10 only at the highest concentrations tested were considered moderate inducers, whereas those with ratios of ≥ 10 at two or more concentrations were considered strong inducers.

Cefoxitin was a strong or moderate inducer for all organisms (Table 1). Both sulbactam and tazobactam were moderate inducers for *A. caviae*, *M. morganii*, and *C. freundii*. However, sulbactam was a moderate inducer for the remaining organisms, whereas tazobactam was only a weak inducer. In contrast, clavulanate was a strong inducer for *M. morganii*, *A. caviae*, and *E. aerogenes* and a moderate inducer for all others. All three companion β-lactams were moderate inducers for *P. aeruginosa*. Cefoperazone and piperacillin were both weak inducers for the remaining organisms. However, ticarcillin was also a moderate inducer for *A. caviae*, *E. aerogenes*, and *S. marcescens* and a strong inducer for *M. morganii* (Table 1).

To extend these observations, the ability of each β-lactamase inhibitor to induce class I enzymes was examined further in disk approximation tests (10) with 10 wild-type strains of each species. Disks containing cefoxitin, clavulanate, sulbactam, or tazobactam were prepared and placed near commercial disks of cefoperazone, piperacillin, and ticarcillin. Disk concentrations for cefoxitin were 15 μg for tests with all species except *S. marcescens* (5 μg) and *Aeromonas* spp. (1 μg). β-Lactamase inhibitors were used at concentrations of 50 μg per disk except for tests with *Aeromonas* spp. (10 μg). The reduced concentration of β-lactamase inhibitors used in tests with *Aeromonas* spp. was due to the potent antibacterial activity of clavulanate against these organisms. Antagonism of β-lactam activity through enzyme induction was detected as a flattening of the zone of inhibition produced by the β-lactam on the side adjacent to the β-lactamase inhibitor or cefoxitin. Figure 1 illustrates a typical disk approximation test demonstrating differential antagonism by the β-lactamase inhibitors and cefoxitin.

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TABLE 1. Individual inducer potencies of three β-lactamase inhibitors and various β-lactam antibiotics

Organism[a]	Inducer	Ratio[b] of induced/uninduced enzyme levels with inducer concn (μg/ml) of:			
		0.1	1	10	100
A. caviae DLS4	Cefoxitin	**28**	**286**		
(0.5 ± 0.1)	Clavulanate		2	**128**	**320**
	Sulbactam		2	6	**96**
	Tazobactam		2	4	**100**
	Cefoperazone	2	2	2	
	Piperacillin	2	2	2	
	Ticarcillin		2	**10**	
C. freundii 21	Cefoxitin		**15**	**117**	
(2.3 ± 0.4)	Clavulanate		1	2	**74**
	Sulbactam		1	1	**14**
	Tazobactam		1	1	**10**
	Cefoperazone		1	1	1
	Piperacillin	1	1	1	
	Ticarcillin	1	1	9	
E. aerogenes 76	Cefoxitin		**18**	**52**	**230**
(7.5 ± 1.0)	Clavulanate		1	**16**	**252**
	Sulbactam		1	1	**12**
	Tazobactam		1	1	4
	Cefoperazone	1	1	1	
	Piperacillin		1	1	1
	Ticarcillin		1	2	**118**
E. cloacae 55	Cefoxitin		**16**	159	
(3.0 ± 0.8)	Clavulanate		1	4	**136**
	Sulbactam		1	1	**15**
	Tazobactam		1	1	6
	Cefoperazone	1	1	2	
	Piperacillin	1	1	1	
	Ticarcillin	1	1	8	
M. morganii 5	Cefoxitin	3	**382**		
(1.0 ± 0.6)	Clavulanate		2	**57**	**469**
	Sulbactam		1	1	**278**
	Tazobactam		1	1	**47**
	Cefoperazone	1	2	3	
	Piperacillin	2	2	9	
	Ticarcillin	1	**18**	**867**	
P. aeruginosa 164	Cefoxitin		5	**51**	**170**
(1.3 ± 0.2)	Clavulanate		**1**	2	**74**
	Sulbactam		1	1	**18**
	Tazobactam		1	1	3
	Cefoperazone		1	3	**33**
	Piperacillin		1	2	**17**
	Ticarcillin		2	5	**50**
S. marcescens 1	Cefoxitin		**72**		
(21.3 ± 3.2)	Clavulanate		1	2	**32**
	Sulbactam		1	1	**13**
	Tazobactam		1	1	4
	Cefoperazone	1	1	2	
	Piperacillin	1	1	2	
	Ticarcillin	1	1	**11**	

[a] Uninduced enzyme levels ± standard deviation (in nanomoles of cephalothin hydrolyzed per minute per milligram of protein) are in parentheses.
[b] Induction ratios of ≥10 are indicated in boldface type.

The results of the disk approximation tests (Table 2) were largely in agreement with the results obtained in the previous quantitative assays (Table 1). As expected, cefoxitin antagonized the activity of the three other β-lactam antibiotics against more than 80% of all strains. Clavulanate antagonized the activity of both piperacillin and ticarcillin against 53% of the strains examined and the activity of cefoperazone against 60% of the strains. In contrast to cefoxitin, clavulanate demonstrated no antagonism in tests with *C. freundii* and *Aeromonas* spp. However, in the latter case this was probably due to the strong antibacterial activity of clavulanate, since this compound was a strong inducer for *A. caviae* DLS4 (Table 1). In general, tazobactam and sulbactam did not antagonize the activity of the three β-lactam antibiotics in disk approximation tests. The sole exception was *Aeromonas* spp., in which both sulbactam and tazobactam antagonized the activity of piperacillin (Table 2).

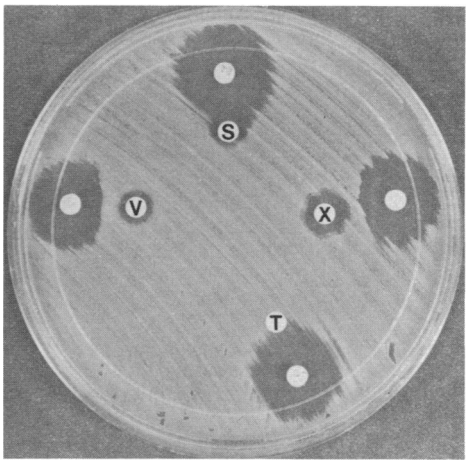

FIG. 1. Disk approximation test with a *M. morganii* strain illustrating differential antagonism by the β-lactamase inhibitors and cefoxitin. Prepared disks of clavulanate (V), sulbactam (S), cefoxitin (X), and tazobactam (T) were placed near commercial disks of piperacillin. A flattening of the zone of inhibition produced by piperacillin disks was interpreted as a positive test for induction by clavulanate and cefoxitin.

Several previous studies have examined the ability of β-lactamase inhibitors to induce class I enzymes among various members of the *Enterobacteriaceae*, showing that clavulanate, but not sulbactam or tazobactam, is a potent inducer of chromosomal β-lactamases (5, 6, 13). Although the results of these studies show some quantitative differences in induction potencies for particular species, these probably reflect differences in bacterial strains and induction parameters (e.g., 2-h versus 4-h exposure to the inducer). As shown here (Table 1), the ability of these compounds to induce chromosomal β-lactamases is also dependent on inducer concentration and probably accounts for the absence of significant induction by clavulanate in studies using only a single, low concentration for induction (1). The use of the disk approximation test (Table 2) to analyze a large number of strains for each species provided a more definitive assessment of the susceptibility of a particular species to induction by these compounds.

It remains uncertain whether the ability of the β-lactamase inhibitors to antagonize the in vitro activity of β-lactam antibiotics will also occur in vivo, as has been seen with cefoxitin (2, 4). Some have claimed that clavulanate, the most potent inducer in vitro, is unlikely to antagonize the efficacy of its companion drug in vivo since concentrations required for induction are well in excess of those achieved clinically (13). However, it should be noted that there are many strain-to-strain variations in the susceptibility of organisms to induction by clavulanate. In addition, since

TABLE 2. Antagonism of β-lactams by three different β-lactamase inhibitors and cefoxitin in disk approximation tests with 10 strains of each species

Organism	β-Lactam	No. of strains showing antagonism with:			
		Cefoxitin	Clavulanate	Sulbactam	Tazobactam
Aeromonas spp.	Cefoperazone	9	2	3	2
	Ticarcillin	7	0	1	1
	Piperacillin	10	1	8	6
C. freundii	Cefoperazone	6	0	1	0
	Ticarcillin	6	1	1	0
	Piperacillin	2	0	0	0
Enterobacter spp.	Cefoperazone	10	7	1	0
	Ticarcillin	10	7	0	2
	Piperacillin	8	4	0	0
M. morganii	Cefoperazone	8	8	0	0
	Ticarcillin	9	9	0	0
	Piperacillin	9	9	0	0
P. aeruginosa	Cefoperazone	9	9	0	0
	Ticarcillin	7	7	0	1
	Piperacillin	10	10	0	0
S. marcescens	Cefoperazone	10	10	0	1
	Ticarcillin	10	8	0	0
	Piperacillin	10	8	0	1
Total	Cefoperazone	52 (87%)[a]	36 (60%)	5 (8%)	3 (5%)
	Ticarcillin	49 (82%)	32 (53%)	2 (3%)	4 (7%)
	Piperacillin	49 (82%)	32 (53%)	8 (13%)	7 (12%)

[a] Percentage of strains tested (60 strains total).

clavulanate is not an efficient inhibitor of class I enzymes (3, 7), its presence in combination with a β-lactam is unlikely to be of benefit when an organism with such an enzyme is encountered. Thus, in view of an unlikely positive effect coupled with a possible negative effect, one might want to consider avoiding combinations containing clavulanate when organisms possessing class I β-lactamases are involved. Similar caveats may also apply to other β-lactam–β-lactamase inhibitor combinations if future studies fail to show any potential positive effects of these combinations.

This study was supported in part by the Health Future Foundation, Omaha, Nebr., and by grants from Lederle, Pearl River, N.Y., and Pfizer-Roerig, New York, N.Y.

We thank Ellen Smith-Moland for technical assistance.

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