

Leakage of β -Lactamase: a Second Mechanism for Antibiotic Potentiation by Amdinocillin

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Discrepancies were observed between results of different beta-lactamase induction tests with amdinocillin, which appeared to be a strong inducer in whole-cell assays but a weak inducer in assays with cell-free sonic extracts. Results of a nitrocephin-disk test with constitutive beta-lactamase producers indicated that the positive results obtained in whole-cell assays were due to drug-produced leakage of enzyme from the cell and not to induction. Imipenem was also found to cause leakage of beta-lactamase from a similar number of constitutive enzyme producers, while cefoxitin was much less likely to cause leakage. A split-dose regimen was employed to treat mice infected with a strain of *Enterobacter cloacae* which appeared to leak enzyme on exposure to amdinocillin. Results indicated that prior treatment with amdinocillin significantly enhanced ($P < 0.025$) the efficacy of azlocillin, an enzyme-labile drug, but did not affect the efficacy of cefotaxime, a relatively enzyme-stable drug. Conversely, prior treatment with amdinocillin did not potentiate the efficacy of either azlocillin or cefotaxime in the treatment of mice infected with an *Escherichia coli* strain that was highly susceptible to all three drugs. Thus, it appears that amdinocillin may potentiate the activity of other beta-lactam drugs not only by binding to a complementary penicillin-binding protein but also by causing leakage of beta-lactamase from the cell. This effect may be related to its ability to bind to penicillin-binding protein 2 and subsequently produce changes in outer membrane permeability.

In a recent study (9), the ability of various beta-lactam antibiotics to induce chromosomal type I beta-lactamases was examined. The results of that study were based on assays performed with sonic extracts prepared from cells harvested after a 2-h exposure to the inducing drug. In preliminary work leading up to the study, induction was assessed initially by using a whole-cell assay. Results from the whole-cell assay suggested that amdinocillin is an inducer of beta-lactamase. However, when assays with cell-free sonic extracts were employed, amdinocillin was found to be a poor inducer. Thus, additional studies were designed to determine why results of induction assays with amdinocillin varied greatly with the method employed.

MATERIALS AND METHODS

Organisms. The organisms used in this study included clinical isolates of bacteria possessing inducible, chromosomally mediated beta-lactamases and their stably derepressed mutants. These have been described previously (9). Bacteria possessing well-characterized beta-lactamases belonging to Richmond and Sykes types I, III, IV, and V were kindly provided by F. Leitner, L. Koupal, and A. Medeiros. These included *Klebsiella pneumoniae* A20346-1 (type IV), *Escherichia coli* A20341-1 (type III), *E. coli* A20343 (type I), *Enterobacter cloacae* 214 (type I), *K. pneumoniae* A20345 (type IV), *K. pneumoniae* A-9679 (type III), *Enterobacter cloacae* P99 (type I), *E. coli* RTEM-R6K (type III), *E. coli* 1752E-RP1 (type III), *E. coli* 1527-RGN238 (type V), *E. coli*

1573-K46 (type V), *E. coli* 1894E-R57b (type V), *E. coli* J532-R997 (type III), and *E. coli* J53-R1010 (type III).

Antibiotics. All antibiotics were obtained as diagnostic powders from their respective manufacturers. Antibiotic disks were either purchased from BBL Microbiology Systems (Cockeysville, Md.) or were obtained from the drug manufacturer.

Susceptibility tests. Agar dilution susceptibility tests were performed in Mueller-Hinton agar (BBL), with an inoculum of 10^5 CFU per spot applied with a Steers replicator (12). The MIC was defined as the lowest concentration preventing visible growth after incubation for 18 h at 35°C in air. The presence of five or fewer colonies was ignored.

Beta-lactamase assays. The ability of various antibiotics to induce beta-lactamase was determined in two assays. In the whole-cell assay, each strain was grown overnight on Mueller-Hinton agar containing antibiotic at one-fourth its MIC or 100 μ g/ml, whichever was less. The strain was then suspended at an inoculum of 10^9 CFU/ml in phosphate buffer (pH 7) containing 100 μ M cephalothin. This was incubated at 37°C for 1 h, during which time there was no loss of cell viability. The cells were then removed by microfiltration, and the concentration of cephalothin in the filtrate was determined by a UV spectrophotometric assay (8). Results were expressed as nanomoles of cephalothin hydrolyzed per 10^9 cells. In the second assay cell-associated beta-lactamase was measured; this assay has been described in detail previously (9). Briefly, each strain was incubated for 2 h at 37°C in Mueller-Hinton broth (BBL) containing antibiotic at one-fourth its MIC or 100 μ g/ml, whichever was less. Cells were harvested and washed twice, and a cell-free sonic extract was prepared. The beta-lactamase activity in these sonicates was determined in a UV spectrophotometric assay

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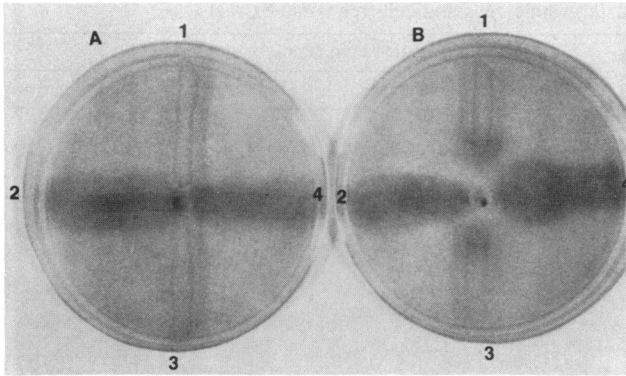


FIG. 1. Nitrocephin-disk test for beta-lactamase leakage. Four different bacterial strains were inoculated in a spoke pattern out from a central paper disk containing no drug (A) and a beta-lactam antibiotic (B). Following overnight incubation, the plates were flooded with nitrocephin. Beta-lactamase was detected as pink areas (appearing as the darker areas in the photograph); this is where the nitrocephin was hydrolyzed. High-level constitutive enzyme producers appeared pink throughout the spoke on control plates (plate A, strains 2 and 4), while low-level constitutive enzyme producers were usually colorless throughout (plate A, strains 1 and 3). An enhanced area of darkness at the end of the spoke next to the drug disk (plate B, strains 1, 3, and 4) indicates leakage of enzyme from the strain.

by using 100 μ M cephalothin as the substrate. Results were expressed as nanomoles of cephalothin hydrolyzed per minute per milligram of protein. In both the whole-cell and cell-free assays, an induction ratio was calculated by using the formula A_i/A_u , where A_i is the beta-lactamase activity following induction, and A_u is the beta-lactamase activity in uninduced cells. This ratio was considered to be indicative of induction if it exceeded the value obtained by dividing the sum of the mean of A_u plus 2 standard deviations (SD) ($\bar{A}_u + 2$ SD) by the mean of A_u (\bar{A}_u).

The extent of leakage of beta-lactamase from viable cells was determined in an assay, as follows. Strains producing beta-lactamase constitutively were incubated with shaking at 37°C for 90 min in 100 ml of Mueller-Hinton broth. Antibiotic was added to a final concentration of one-fourth the MIC or 100 μ g/ml, whichever was less, and incubation was continued for an additional 2 h. Cells were harvested by centrifugation, and sonic extracts were prepared in the same manner as described above for the cell-free beta-lactamase assay. The supernatant of the culture was dialyzed overnight at 4°C and concentrated 100-fold by using a MicroProDiCon apparatus (Bio-Molecular Dynamics, Beaverton, Ore.). Protein in both the sonic extracts and concentrated supernatants was determined by the method described by Bradford (2) by using a protein assay kit (Bio-Rad Laboratories, Richmond, Calif.). The beta-lactamase activity in both the sonic extracts and concentrated supernatants was determined in spectrophotometric assays with nitrocephin. The color of the concentrated supernatants necessitated the use of nitrocephin in these assays. Results obtained with the sonicates were expressed as nanomoles of nitrocephin hydrolyzed per minute per milligram of protein. Because the protein content of the concentrated supernatants was similar and reflected, for the most part, the protein of the growth medium and not cellular protein, results were expressed as nanomoles of nitrocephin hydrolyzed per minute per milliliter.

Nitrocephin-disk test. The ability of an antibiotic to produce leakage of beta-lactamase was determined in an assay,

as follows. An antibiotic disk was placed in the center of a Mueller-Hinton agar plate, and strains were inoculated in a spoke pattern out from the disk (Fig. 1). This plate was incubated overnight. The plate was then flooded with a solution of 500 μ g of nitrocephin per ml, and results were read within 2 min. Enhanced beta-lactamase at the end of the spoke closest to the disk appeared as an enlarged pink area in comparison with the end of the spoke away from the disk (Fig. 1). For constitutive enzyme producers, this enhanced beta-lactamase activity represented drug-produced leakage from the cell.

Split-dose regimen. Experimental infections were induced in male CF-1 mice (body weight, 20 to 25 g) by intraperitoneal inoculation of 1 minimal lethal dose for 100% of the mice. The number of viable bacteria in the challenge was measured by dilution plate counts. At 1 and 3.5 h postinfection, amdinocillin, azlocillin, or cefotaxime was administered intramuscularly at five to six different dosages to groups of mice (10 mice per group). An infected but untreated group of mice served as a control for the virulence of the bacterial challenge. The dose (in milligrams per kilogram) required to protect 50% of mice from death (PD_{50}) through the 48-h postchallenge period was determined by log probit plot, and the 95% confidence levels were calculated (4).

To examine the effect of drug combinations and sequencing, each antibiotic was used in a split-dose regimen at biologically equivalent concentrations, i.e., the PD_{50} . Eight groups of 20 animals each were used in this regimen. One group each received amdinocillin at 1 h postchallenge and azlocillin or cefotaxime at 3.5 h postchallenge. Two other groups received azlocillin or cefotaxime at 1 h postchallenge and amdinocillin at 3.5 h postchallenge. Because the PD_{50} for any antibiotic is a calculated value, the actual protection provided by this dose was determined with three additional groups of animals which received azlocillin, amdinocillin, or cefotaxime at 1 and 3.5 h postchallenge. The eighth group was an untreated control group. Thus, in this split-dose regimen, animals treated with two different antibiotics received total drug that was biologically equivalent to one drug alone. This design allowed one antibiotic to be substituted for, rather than added to, the second antibiotic. Differences in survival between the various groups were compared by using the chi-square test with the Yates correction factor.

RESULTS

Beta-lactamase induction. Initial beta-lactamase induction tests were performed on six strains of gram-negative bacteria, each possessing an inducible, chromosomally mediated type I beta-lactamase. The ability of cefoxitin, imipenem, amdinocillin, and ceftriaxone to induce beta-lactamase in these strains was determined in assays with whole-cell and cell-free sonic extracts. In both assays, cefoxitin and imipenem induced beta-lactamases in all six strains (Table 1). In contrast, ceftriaxone showed little or no inducer activity in either assay. Amdinocillin appeared to induce beta-lactamase in whole-cell assays with *Serratia liquefaciens* 36, *Morganella morganii* 5, and *Pseudomonas aeruginosa* 164. When cell-free sonic extracts of these organisms were examined, however, amdinocillin was generally inactive or weak as an inducer.

Tests for leakage of beta-lactamase. Because results of whole-cell assays would be falsely elevated if the beta-lactamase were to leak from the cell during the test, the

TABLE 1. Induction of beta-lactamase in assays by using whole cells and cell-free sonic extracts

Assay type and strain	Uninduced enzyme activity	Induction ratio after exposure to ^a :			
		Cefoxitin	Imipenem	Amdinocillin	Ceftriaxone
Whole cells					
<i>Serratia liquefaciens</i> 36	53 ± 6 ^b	1.5 ^c	1.5 ^c	1.5 ^c	1.1
<i>Enterobacter cloacae</i> 55	46 ± 5 ^b	1.6 ^c	1.4 ^c	1.0	1.2
<i>Citrobacter freundii</i> 21	31 ± 6 ^b	1.8 ^c	1.7 ^c	0.8	0.8
<i>Serratia marcescens</i> 1	18 ± 8 ^b	2.6 ^c	2.3 ^c	1.6	1.3
<i>Morganella morganii</i> 5	21 ± 5 ^b	3.6 ^c	3.9 ^c	3.1 ^c	1.5
<i>Pseudomonas aeruginosa</i> 164	6 ± 4 ^b	14.2 ^c	9.5 ^c	4.5 ^c	0.7
Cell-free sonic extracts					
<i>Serratia liquefaciens</i> 36	14 ± 6 ^d	152 ^c	277 ^c	2	1
<i>Enterobacter cloacae</i> 55	15 ± 6 ^d	46 ^c	40 ^c	2	1
<i>Citrobacter freundii</i> 21	28 ± 8 ^d	14 ^c	8 ^c	1	1
<i>Serratia marcescens</i> 1	46 ± 15 ^d	50 ^c	8 ^c	2	1
<i>Morganella morganii</i> 5	11 ± 4 ^d	48 ^c	91 ^c	2 ^c	3 ^c
<i>Pseudomonas aeruginosa</i> 164	13 ± 3 ^d	25 ^c	47 ^c	2 ^c	1

^a Enzyme activity after induction/enzyme activity before induction.

^b Nanomoles of cephalothin hydrolyzed per 10⁹ cells; $\bar{A}_u \pm SD$.

^c Ratio above $(\bar{A}_u + 2 SD)/\bar{A}_u$.

^d Nanomoles of cephalothin hydrolyzed per minute per milligram of protein; $\bar{A}_u \pm SD$.

positive results obtained with amdinocillin in these assays could have been due to the fact that the drug caused the cells to leak their periplasmic contents rather than to true enzyme induction. To test this possibility, leakage of beta-lactamase from 20 strains producing enzyme constitutively was examined in the nitrocephin-disk test by using amdinocillin, cefoxitin, and imipenem. Enzyme leakage was detected with 10, 9, and 5 of the 20 strains in tests with amdinocillin, imipenem, and cefoxitin, respectively (Table 2).

The ability of amdinocillin, imipenem, and cefoxitin to cause leakage of beta-lactamase was examined directly in tests with *E. coli* M128 (SHV-1) and *Enterobacter cloacae* 55M (type I). These strains were chosen because the nitrocephin-disk test was positive for all three drugs with the former strain and negative for all three drugs with the latter strain. Each strain was incubated for 2 h in broth containing drug at one-fourth its MIC. At the end of this time, the beta-lactamase activity in the cells and culture supernatant was determined by using nitrocephin as the substrate, as described above. The beta-lactamase activity in the supernatant of *E. coli* M128 was much lower than that found in the cells (Table 3). Exposure of this strain to amdinocillin, imipenem, and cefoxitin raised the activity in the culture supernatants. In contrast, no increase in the beta-lactamase activity of the supernatants of cultures of *Enterobacter cloacae* 55M was found following exposure of the strain to any of the three drugs.

In vivo studies. The apparent ability of amdinocillin to

TABLE 2. Leakage of beta-lactamases from constitutive enzyme producers, as detected in the nitrocephin-disk test

Beta-lactamase type produced ^a	No. of strains tested	No. of strains showing leakage of beta-lactamase in tests with:		
		Amdinocillin	Imipenem	Cefoxitin
I	9	5	2	0
III	6	3	6	5
IV	2	1	1	0
V	3	1	0	0

^a Richmond and Sykes beta-lactamase type.

cause leakage of beta-lactamases without inducing the enzymes suggested that prior treatment with this drug might render organisms more susceptible to enzyme-labile drugs. To test this hypothesis, mice were infected with *Enterobacter cloacae* 20, a murine-virulent strain with an inducible type I beta-lactamase (2 and 691 nmol of cephalothin hydrolyzed per min per mg for uninduced and cefoxitin-induced cells, respectively). In nitrocephin-disk tests this strain appeared to leak enzyme when exposed to amdinocillin. The infected mice were then treated with amdinocillin, azlocillin, or cefotaxime in the split-dose regimen to determine if prior treatment with amdinocillin enhanced the efficacy of the other two drugs. Azlocillin was chosen as one companion drug because it, like other penicillins (9), was found to be a poor inducer of type I beta-lactamases, did not cause leakage of beta-lactamase in *Enterobacter cloacae* 20 (data not shown), and is relatively enzyme labile. Cefotaxime was chosen as the second companion drug because it, too, was a poor inducer; did not cause enzyme leakage; and is relatively enzyme stable. Treatment with amdinocillin followed by treatment with azlocillin significantly improved protection over that provided by amdinocillin alone, azlocillin alone, or

TABLE 3. Leakage of beta-lactamase from constitutive enzyme producers measured directly

Isolate (beta-lactamase type)	Isolate grown in broth containing	Beta-lactamase activity in:	
		Cells ^a	Supernatant ^b
<i>Escherichia coli</i> M128 (III)	No drug	295	15
	Amdinocillin	214	54
	Imipenem	250	41
	Cefoxitin	197	37
<i>Enterobacter cloacae</i> 55M (I)	No drug	7,798	109
	Amdinocillin	7,608	47
	Imipenem	8,575	47
	Cefoxitin	6,291	109

^a Activity expressed as nanomoles of nitrocephin hydrolyzed per minute per milligram of protein.

^b Activity expressed as nanomoles of nitrocephin hydrolyzed per minute per milliliter of supernatant concentrate.

TABLE 4. In vivo efficacy of beta-lactam combinations in treatment of infections caused by gram-negative bacilli^a

Group	Antibiotic administered at:		Outcome (%) of infection with: ^b	
	1 h postchallenge	3.5 h postchallenge	<i>Enterobacter cloacae</i> 20	<i>Escherichia coli</i> 3
1	Azlocillin	Azlocillin	5/20 (25)	6/20 (30)
2	Amdinocillin	Amdinocillin	4/20 (20)	5/20 (25)
3	Azlocillin	Amdinocillin	5/20 (25)	7/20 (35)
4	Amdinocillin	Azlocillin	14/20 (70) ^c	7/20 (35)
5	Cefotaxime	Cefotaxime	6/20 (30)	15/20 (75)
6	Cefotaxime	Amdinocillin	6/20 (30)	8/20 (40)
7	Amdinocillin	Cefotaxime	7/20 (35)	8/20 (40)
8	None	None	0/20 (0)	0/20 (0)

^a The beta-lactam dose used was 25.3 and 13.2 mg/kg for azlocillin, 0.72 and 0.2 mg/kg for amdinocillin, and 0.11 and 0.08 mg/kg for cefotaxime for *Enterobacter cloacae* 20 and *E. coli* 3, respectively. MICs were 8 and 4 μ g/ml for azlocillin, 0.12 and 0.06 μ g/ml for amdinocillin, and 0.12 and 0.12 μ g/ml for cefotaxime for *Enterobacter cloacae* 20 and *E. coli* 3, respectively.

^b Number of animals alive/number of animals infected.

^c Significantly higher than regimen 2 ($P < 0.005$) and regimens 1 and 3 ($P < 0.025$ by the chi-square test with Yates correction). No other differences were statistically significant.

azlocillin administration prior to amdinocillin administration (Table 4). Treatment with amdinocillin prior to treatment with cefotaxime did not improve the efficacy of cefotaxime. This in vivo potentiation of azlocillin by amdinocillin appeared to be due primarily to leakage of beta-lactamase because potentiation of cefotaxime should have been observed if it were due to a generalized increased permeability caused by amdinocillin. To test this aspect directly, however, the split-dose regimen was used to treat mice infected with *E. coli* 3. This strain possessed no plasmid-mediated beta-lactamase and was highly susceptible to all study drugs. There was no potentiation of either azlocillin or cefotaxime by amdinocillin in tests with *E. coli* 3 (Table 4).

Relationship between induction and leakiness. Cell-free assays for induction of beta-lactamase and nitrocephin-disk tests were performed with a broad array of beta-lactam drugs to determine their propensity to cause induction, leakiness, or both. The six strains with inducible beta-lactamases that had been examined initially were used in these tests. Good inducers like cefoxitin, cefmetazole, and imipenem usually gave positive results in both cell-free assays and nitrocephin-disk tests (Table 5). The penicillins, the expanded spectrum cephalosporins, and aztreonam generally gave negative results in both tests.

DISCUSSION

The leakage of periplasmic beta-lactamase from amdinocillin-treated cells observed in this study strongly suggests a change in outer membrane permeability. This could directly enhance the activity of a second beta-lactam drug by a number of mechanisms. The activity of enzyme-labile drugs would be enhanced because of the loss of enzyme from the cell. This enhancement would be greatest for cells producing very little enzyme. The activity of both enzyme-labile and -stable drugs could also be potentiated through increased penetration into the cell. This type of enhancement would be greatest for drugs for which the outer membrane represented the largest penetration barrier. Thus, it would be difficult to predict a priori the precise effect that amdinocillin should have on a second beta-lactam drug. The quantity and type of beta-lactamase present would need to be assessed, as would

the ability of amdinocillin to cause leakage. The enzyme lability and penetrability of the second drug would also have to be known before any accurate predictions of potentiation could be made.

Because there appear to be numerous mechanisms whereby amdinocillin can enhance the activity of a second beta-lactam, some of which involve direct effects on penicillin-binding protein (PBP) 2 and others of which involve permeability, it is not surprising that it has been difficult to design an in vitro test that is predictive of synergy in vivo (3). It is unlikely that the ability of amdinocillin to produce leakage will, by itself, be a better predictor of in vitro or in vivo synergy as it is now defined and tested. In this study, however, the ability of amdinocillin to produce leakage of beta-lactamase from a strain possessing low levels of enzyme was predictive of in vivo enhancement of the efficacy of a relatively poorly penetrating enzyme-labile drug like azlocillin. Lack of a strong permeability barrier to cefotaxime in *Enterobacter cloacae* 20 and to azlocillin and cefotaxime in *E. coli* 3 probably explains the inability of amdinocillin to enhance activity of these drugs in vivo. The ability of amdinocillin to potentiate the activity of other beta-lactams against organisms that are resistant to the drugs because of outer membrane impermeability, however, needs to be assessed directly.

The ability of amdinocillin to produce leakage of beta-lactamase may be related to its ability to bind PBP 2 (10). This is supported by the observation that those beta-lactams listed in Table 5, which, like amdinocillin, bind to PBP 2 (i.e., Sch 34343, imipenem, and potassium clavulanate), were more likely to cause leakage than those which do not bind to PBP 2. An analysis of the number of these showing leakage of beta-lactamase in the absence of enzyme induction (i.e., positive nitrocephin-disk tests but negative cell-free assays) supports this contention. Of eight tests in which no induction occurred with drugs that bind PBP 2, leakage was shown in seven (88%) tests. In contrast, there were 54 tests in which no induction occurred with drugs that do not bind PBP 2. In only 15 (28%) of these 54 tests was there evidence of leakage. This difference between the two groups

TABLE 5. Relationship between induction of beta-lactamase and results of nitrocephin-disk tests with six strains of gram-negative bacilli^a

Antibiotic	No. of strains showing ^b :					
	I ⁺ /N ⁺	I ⁺ /N ⁻	I ⁻ /N ⁺	I ⁻ /N ⁻	I ⁺	N ⁺
Cefoxitin	5	1			6	5
Cefmetazole	6				6	6
Imipenem	6				6	6
Sch 34343	4		2		4	6
Potassium clavulanate	4		1	1	4	5
Amdinocillin	1	1	4		2	5
Sulbactam		1	2		1	2
Cephalothin		3			3	0
Cefamandole	1	3			4	1
Cefotaxime		1	2		1	2
Moxalactam			1		0	1
Cefoperazone		1	2		1	2
Ceftazidime		1	2		1	2
BMV-28142			2		0	2
Aztreonam		1			1	0
Carbenicillin			2		0	2
Mezlocillin			2		0	2

^a Tests were performed on the same strains listed in Table 1.

^b I⁺ and I⁻ indicate results of cell-free assay for induction of beta-lactamase; N⁺ and N⁻ indicate results of nitrocephin-disk test.

of drugs was significant (chi-square with Yates correction, 8.4; $P < 0.005$). Further support for a propensity of drugs that bind PBP 2 to cause leakage is provided by results of a recent report (1). These investigators found that potassium clavulanate could potentiate the activity of ceftazidime against a strain of *E. coli* that was resistant to ceftazidime because of altered outer membrane permeability. This potentiation of ceftazidime by potassium clavulanate was shown to be due to ability of potassium clavulanate to enhance drug penetration via alteration of outer membrane proteins.

The greater propensity of drugs that bind PBP 2 to cause leakage may be explained best by results of studies with amdinocillin, which is somewhat unique in its ability to bind solely to PBP 2 (10). The major direct effect of amdinocillin is to produce osmotically stable spherical cells which, in the proper environment, lyse because of increased cell mass without cell division (11). Both growth inhibition and lysis are prevented by high salt concentrations, but induction of spherical cells remains unaltered (11). These spherical cells are hypersensitive to other beta-lactam antibiotics which bind to PBPs 1, and 3, or both (11). This hypersensitivity has been thought to be due directly to the synergistic effects of binding to complementary PBPs. Results of this study and observations made with amdinocillin-resistant mutants, however, suggest that a change in permeability may also be involved.

Certain types of amdinocillin-resistant mutants have a defective PBP 2 and grow as osmotically stable spherical cells (5, 7, 13). These tend to be hypersensitive to other beta-lactam antibiotics as well as to dyes and detergents that are normally excluded by the outer membrane (6, 14-16). These observations also suggest that impairment of PBP 2 via mutation may cause alterations in the function of the outer membrane. Although Matsuhashi et al. (6) reported slight changes in membrane proteins of such mutants and in wild-type strains treated with amdinocillin, they did not examine outer membrane proteins specifically. In other studies in which permeability or specific changes in outer membrane proteins were examined, spherical mutants that were not resistant to amdinocillin (16) or amdinocillin-resistant mutants that were not spherical nor hypersensitive to other beta-lactams, dyes, or detergents were tested (1a). Thus, direct assessment of changes in the outer membrane and permeability still remains to be performed.

Additional studies will be needed to assess the role that altered permeability plays in the interaction of amdinocillin with another beta-lactam antibiotic. The propensity of other drugs that also bind PBP 2 to produce leakage of periplasmic contents should also be assessed. Nevertheless, it is clear from the results of this study that amdinocillin is capable of enhancing a second beta-lactam antibiotic by mechanisms other than binding to complementary PBPs. The results of this study also suggest that if a permeabilizing effect can be produced predictably, sequential administration of certain beta-lactam combinations may represent a promising approach for maximizing the efficacy of these antibiotics.

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