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β-Lactamase Inhibitors from Laboratory to Clinic

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INTRODUCTION

Bacteria are remarkably adaptable organisms that possess an almost unlimited capability to survive under adverse conditions. One of the most effective survival mechanisms among pathogenic bacteria is the production of β-lactamases, enzymes that destroy β-lactam antibiotics.

Although β-lactamase activity can be detected in most strains of gram-negative and gram-positive bacteria (123) as well as in yeasts (100), blue-green algae (cyanobacteria) (89), and mammalian kidney (88), some of these enzymes bear only superficial resemblances to each other. In this review only those β-lactamases produced by bacteria are considered. Even within this limited group great diversity is seen, resulting in major clinical problems caused by bacterial β-lactamases.

Less than 10 years after the clinical introduction of penicillins, penicillin-resistant *Staphylococcus aureus* was observed in a majority of gram-positive infections. The initial response by the pharmaceutical industry was to develop β-lactam antibiotics that were stable to the specific β-lactamases secreted by *S. aureus*. However, as a result, bacterial strains producing β-lactamases with different properties were selected. This cycle of resistance counteracting resistance continues even today.

Within the last 12 years potent β-lactamase inhibitors such as clavulanic acid and sulbactam have become available for halting the action of common β-lactamases. In spite of the effectiveness of some of these inhibitors in vitro, their success has not always resulted in protection of hydrolyzable β-lactam antibiotics in vivo. As with the "β-lactamase-stable antibiotics," a single inhibitor is not always effective for all of the different β-lactamases that may occur in mixed infections.

Although β-lactamase inhibitors have been reviewed extensively over the last 5 years (33, 41, 86), this review intends to emphasize the clinical aspects of β-lactamase inhibitors. Following a description of the enzymology involved in inhibiting β-lactamases, the importance of these molecules in treating infections is addressed.

β-LACTAMASES

Production Characteristics

β-Lactamases can be found either extracellularly or within the periplasmic space. In general, active β-lactamases from gram-positive bacteria are excreted into the medium. β-Lactamase activity in gram-negative organisms is found primarily in the periplasmic space, although some leakage of enzyme into the medium can occur.

Genetic information for β-lactamase synthesis either can be carried on a plasmid or can occur within the bacterial chromosome; either of these can result in the production of enzymes leading to resistance to the common β-lactam antibiotics.

Plasmid-mediated β-lactamases are especially insidious because of the ease with which these extrachromosomal elements can be transferred from one bacterial strain to another. Some β-lactamases, initially coded for on a plasmid, can have this genetic information eventually incorporated into the chromosome as a permanent addition to the cellular deoxyribonucleic acid. It is not unusual for bacteria to carry multiple plasmids, coding for multiple antibiotic-modifying enzymes. It is also possible that multiple resistance factors can be carried on a single plasmid. Thus, it is becoming common for bacteria to appear with resistance to two or three classes of antibiotics.

Chromosomally mediated β-lactamases pose a different situation. Although it has been proposed that all bacteria possess the ability to produce β-lactamase activity (99), in some strains this activity is basically undetectable under normal growth conditions. However, in other strains extraordinarily high levels of β-lactamase may be observed, either with or without induction.

One of the most troubling aspects of chromosomal β-lactamase production is the ease of inducibility of these enzymes, resulting in high concentrations of β-lactamase. The best inducers known are β-lactam antibiotics, frequently those that are subsequently hydrolyzed by the induced enzyme. In some cases a stably derepressed mutant may be selected, with total β-lactamase content representing as much as 4% of the total protein in the bacterial cell (37).

TABLE 1. Substrate profiles for TEM-2, a broad-spectrum β-lactamase, and P99, a cephalosporinase

Substrate	k_{cat} (s^{-1})	
	TEM-2[a]	P99[b]
Penicillin		
Benzylpenicillin	1,030	56
Ampicillin	900	0.15[c]
Carbenicillin	57	ND[d]
Piperacillin	1,140	1.8
Ticarcillin	130	ND
Cephalosporin		
Cephaloridine	1,200	1,000
Cefoperazone	140	8.9
Cephalothin	120	170[c]
Cefaclor	61[c]	140[c]
Ceftriaxone	1.1[c]	0.036[c]
Cefotaxime	0.93	0.13
Ceftazidime	0.01	0.002[e]
Cefoxitin	0.004	0.037[c]
Others		
Aztreonam	2.2	0.00002[e]
Imipenem	0.009[c]	0.002[e]

[a] Reference 34.
[b] Reference 35.
[c] Bush, unpublished data.
[d] ND, Not determined.
[e] Reference 37.

Substrate Specificities

β-Lactamases destroy β-lactam antibiotics by catalyzing the hydrolysis of the β-lactam ring, thereby rendering the antibiotic ineffective (Fig. 1). The reaction may be represented by the following equation:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \cdot S \xrightarrow{k_2} E\text{-}S \xrightarrow{k_3} E + P \quad (1)$$

where E is β-lactamase, S is a β-lactam substrate, $E \cdot S$ is a Michaelis complex formed between enzyme and substrate, E-S is a covalent enzyme-substrate complex (acyl enzyme), and P is the hydrolyzed β-lactam reaction product.

The rate at which hydrolysis occurs (k_3, or k_{cat}) can exceed values of 1,000 molecules of antibiotic hydrolyzed per second per molecule of enzyme, or it can be <0.1 molecule of antibiotic hydrolyzed per hour per molecule of enzyme (Table 1). Every β-lactamase exhibits its own range of hydrolysis rates for specific antibiotics. Hydrolysis rates for standard β-lactam antibiotics are given in Table 1 for two common β-lactamase types.

Because of the variety of β-lactamases in existence, it has become necessary to develop criteria by which these enzymes can be compared. One of the major characteristics that can be used to differentiate classes of β-lactamases is the "substrate profile" for each enzyme. Substrate profiles can be based on a variety of parameters related to the rate at which hydrolysis of specific β-lactams occurs (35). Any of these profiles can be used for evaluation as long as comparable experimental parameters are well defined for that set of data.

One usable classification scheme (130, 139), based upon general substrate specificity, includes penicillinases, enzymes that prefer to hydrolyze penicillins; cephalosporinases, enzymes that preferentially hydrolyze cephalosporins; and broad-spectrum β-lactamases, enzymes that can hydrolyze both selected penicillins and cephalosporins. Within each of these major headings, subgroups can be formed based upon isoelectric points, inhibitor profiles, and ability to hydrolyze specific substrates.

Another scheme is that of Ambler (6), in which β-lactamases are assigned to class A, B, or C according to molecular structure. At the present time, this scheme will work only for well-studied, highly purified β-lactamases which have undergone sequencing or crystallographic evaluation. Ideally, when gene sequencing or amino acid sequence determinations become routinely available for any enzyme in question, this method of classification will surely be the classification scheme of choice.

One of the most commonly used classification schemes is that of Richmond and Sykes (123). This scheme was proposed in 1973 for β-lactamases described at that time in gram-negative bacteria. By using information based on substrate profiles, isoelectric point, and inhibition data, β-

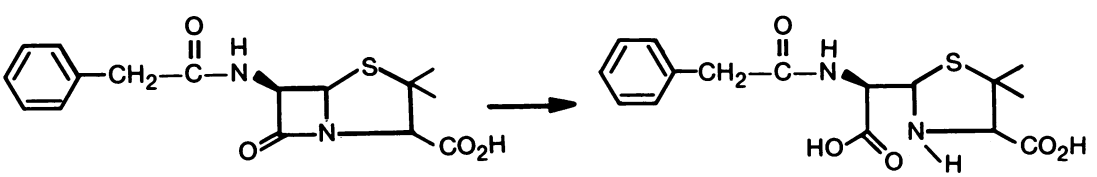

FIG. 1. Hydrolysis of benzylpenicillin by a β-lactamase.

lactamases were grouped into classes I to V. However, this scheme is not as encompassing as the others described above. β-Lactamases produced by gram-positive bacteria are not included, and certain "new" enzymes such as the zinc-containing β-lactamase from *Pseudomonas maltophilia* do not fit into any of the classes described (19).

A short summary of representative β-lactamases of clinical importance is shown in Table 2. It is interesting to note that class A β-lactamases include those enzymes that hydrolyze penicillins or are considered broad-spectrum enzymes. Class C enzymes appear to have substrate profiles corresponding to cephalosporinases. However, the most important feature of this compilation is the variety of β-lactamases that occur in clinical settings. As will be seen throughout this discussion, the diversity of β-lactamases is a most critical aspect of antimicrobial therapy.

β-LACTAMASE INHIBITION

Mechanisms of Action: General

Enzyme inhibitors can be classified as either reversible or irreversible. The determination of class of inhibitor is important in that it will give some indication as to the permanence of the effect of inhibition. A schematic diagram depicting the two classes of β-lactamase inhibitors is shown in Fig. 2.

Reversible inhibitors are those that bind to an enzyme in such a manner that enzyme activity may be restored. The following equation illustrates the equilibrium established between an enzyme (E) and a reversible, noncovalent inhibitor (R):

$$E + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \cdot R \qquad (2)$$

Because this is a dynamic equilibrium, inhibition may be diminished by diluting the inhibitor or by providing another molecule that binds to the enzyme at the same place as the inhibitor.

Inhibitors of β-lactamases that bind at or close to the active site are often β-lactams. Although these molecules may act as inhibitors, they also can be hydrolyzed as substrates. Thus, many reversible inhibitors are really poor substrates that are bound with high affinity but are hydrolyzed at low rates. These would be represented as *E-I* in equation 3 in a form analogous to the *E-S* complex in equation 1, resulting in free enzyme (E) and a hydrolyzed inhibitor (P).

These reversible inhibitors may be characterized by an equilibrium constant, K_i, a value equal to the ratio of rate constants k_{-1}/k_1 (equation 1 or 2). K_i can be readily determined experimentally by a variety of kinetic methods (29). A K_i value is independent of substrate concentration and represents the affinity of the inhibitor for the enzyme.

Irreversible inhibitors may be more effective than reversible inhibitors in that the eventual result is destruction of enzymatic activity. Irreversible inhibitors usually require a finite time period in which to work. This time dependence for inhibition is the result of the following series of reactions:

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \cdot I \overset{k_2}{\rightarrow} E\text{-}I \overset{k_3}{\rightarrow} E\text{-}I^* \qquad (3)$$

where E is the enzyme, I is the inhibitor, $E \cdot I$ is a reversible complex that can dissociate to free enzyme and inhibitor or can proceed to form a covalent complex (acyl enzyme), *E-I*, and *E-I** represents an inactivated enzyme that is not capable of processing substrate. The preferred terminology for

TABLE 2. Classification schemes for β-lactamases

β-Lactamase group	Organisms	Representative enzymes	Plasmid mediated	Inducible	Substrates hydrolyzed[a]	Metal ion	Richmond and Sykes class[b]	Molecular class[c]
Gram positives	*Staphylococcus aureus*	PC1	+	+	Pen		NA[d]	A
	Bacillus licheniformis		−	+	Pen		NA	A
	Bacillus cereus	I	−	+	Pen		NA	A
	B. cereus	II	−	+	Ceph	Zn^{2+}	NA	B
Cephalosporinases	*Enterobacteriaceae*	P99	−	+, −	Ceph		I	C
	Pseudomonas aeruginosa	Sabath and Abraham	−	+	Ceph		I	C
	Escherichia coli	ampC	−	+	Ceph		I	C
Broad-spectrum TEM types	*Enterobacteriaceae*	TEM-1, 2, SHV-1, HMS-1	+	−	Pen, Ceph		III	A
	Klebsiella spp.	K1	−	−	Pen, Ceph		IV	A
Carbenicillin hydrolyzing	*P. aeruginosa* *E. coli* *Shigella* spp. *Salmonella* spp.	PSE-1-4	+	−	Pen, Carb		V	ND[e]
Cloxacillin hydrolyzing	*Enterobacteriaceae*	OXA-1–7	+	−	Pen, Clox		V	ND
Penem hydrolyzing	*Pseudomonas maltophilia*	L1	−	+	Pen, Penem	Zn^{2+}	ND	ND

[a] Pen, Penicillins; Ceph, cephalosporins; Carb, carbenicillin; Clox, cloxacillin; Penem, carbapenems.
[b] Reference 123.
[c] Reference 6.
[d] NA, Not applicable.
[e] ND, Not determined.

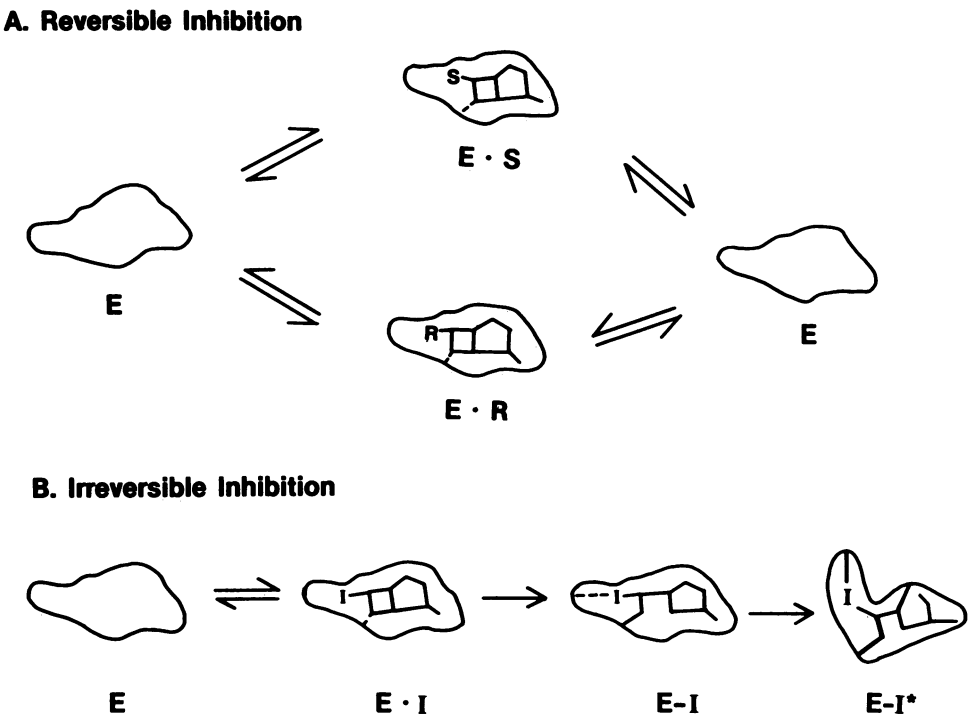

FIG. 2. Schematic representations of reversible and irreversible inhibitors of β-lactamases. (A) Reversible inhibition in which $E \cdot R$ is the enzyme complex formed with a reversible, competitive inhibitor and $E \cdot S$ is the reversible enzyme-substrate complex. (B) Irreversible inhibition in which $E \cdot I$ represents a reversible enzyme-inhibitor complex, *E-I* is a covalent enzyme-inhibitor complex, and *E-I** is the irreversibly inactivated enzyme.

inhibitors that render their targets useless is "inactivators." A special subclass is the suicide inactivator, a molecule that must bind initially at the enzyme-active site, but which is converted into an inactivator through catalytic action of the enzyme itself (1).

Mechanisms of Action: Specific

Specific inhibitors of β-lactamases include the clavam clavulanic acid, the penicillanic acid sulfones sulbactam and YTR 830, 7-acetylmethylenepenicillanic acid (AMPA), and the monobactams aztreonam and SQ 27,327. Of these molecules, only clavulanic acid and sulbactam (Fig. 3) are currently used clinically to protect β-lactamase-susceptible antibiotics.

Clavulanic acid. Clavulanic acid was the first suicide inactivator of β-lactamases to be described in the literature (24). This natural product was isolated from *Streptomyces clavuligerus* on the basis of its potent inhibitory activity against the broad spectrum β-lactamase from *Klebsiella pneumoniae*. The clavam structure was the first naturally occurring bicyclic β-lactam described that did not possess a penicillin or cephalosporin nucleus.

Further studies with additional β-lactamases indicated that clavulanic acid was quite effective in preventing the destruction of substrates of enzymes that could effectively hydrolyze penicillins (Table 3). Inhibition of cephalosporinases from a variety of sources was considerably weaker.

The mechanism for inactivation was studied in detail for the PC1 (40), TEM-2 (42, 60), *Bacillus cereus* I (52), K1, and *Proteus mirabilis* (121) β-lactamases. Although interaction of clavulanate with these β-lactamases all produced the same end result, enzyme inactivation, the individual mechanisms varied somewhat among the different systems.

Results from Knowles and co-workers provided the first evidence that clavulanic acid behaved like a suicide inactivator (42, 60). The exact mechanism by which this occurred was shown to be quite complex, with multiple forms of inhibited enzyme present after initial binding of inhibitor to the TEM-2 β-lactamase. Once an acyl enzyme *E-I* was formed, there were several potential fates for this entity:

$$E\text{-}I \begin{cases} \rightleftharpoons E\text{-}T \\ \rightarrow E + P \\ \searrow E\text{-}I^* \end{cases} \quad (4)$$

The acyl enzyme could yield a transiently inhibited form, *E-T*, which is not permanently inactivated. This type of inhibition would be similar to reversible inhibition in that inhibition can be reversed with addition of substrate and free enzyme can eventually be recovered. A second possibility is that the acyl enzyme can simply act as an enzyme-substrate complex, with eventual hydrolysis of clavulanate (*P*), again resulting in the release of free enzyme (*E*). A third alternative was irreversible inactivation (Fig. 2B). In this scenario the end result (*E-I**) is permanent loss of enzymatic activity, a desirable property for a bacterial enzyme inhibitor.

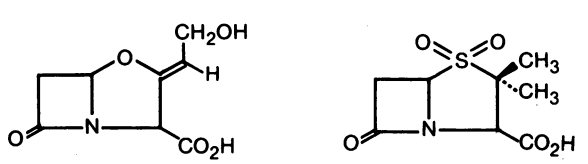

FIG. 3. Structures of clinically approved β-lactamase inactivators.

TABLE 3. Inhibition of β-lactamases by clavulanic acid

β-Lactamase	Type[a]	I_{50} (μg/ml)	Reference
Enterobacter cloacae P99	Cephase	110	23
Proteus mirabilis	Penase	0.015	23
TEM-1	Broad spectrum	0.06	23
Klebsiella pneumoniae K1	Broad spectrum	0.007	23
Staphylococcus aureus	Penase	0.03	23
Branhamella catarrhalis Ravasio	Broad spectrum	0.04	56
B. catarrhalis 1908	Broad spectrum	0.10	56
Serratia marcescens	Cephase	39	73
Morganella morganii	Cephase	30	73

[a] Cephase, cephalosporinase; penase, penicillinase; broad spectrum, broad-spectrum β-lactamase.

As can be seen from this set of interactions, the extent to which inactivation occurs depends upon the interrelationships among the various pathways. The effectiveness of an inhibitor will depend upon the relative ratios of formation of *E-I** compared with *E-T* or *E* + *P*. As indicated above, experimental evidence supported the existence of all three pathways for the interaction of TEM-2 β-lactamase with clavulanic acid. In fact, a more complicated picture was presented with this enzyme in that multiple forms of transiently inhibited enzyme were present. Eventually, however, the end result of this kind of inhibitory action is the observation of loss of enzymatic activity.

It is possible to evaluate the effectiveness of such an inactivator experimentally by determining the number of molecules of inhibitor that are hydrolyzed (on the average) by a single enzyme molecule before irreversible inactivation occurs. Inhibition of the TEM-2 β-lactamase occurred after consumption of 115 molecules of clavulanate (Table 4) (60), indicating that at least a 100-fold excess of clavulanic acid over enzyme would be required before substantial inhibition of enzyme activity is observed. The *S. aureus* PC1 β-lactamase was inhibited most effectively, with a 1:1 ratio of inhibitor/enzyme resulting in complete inactivation of the β-lactamase (40). Thus, the alternative pathways leading to transiently inhibited forms or to hydrolyzed clavulanate are not important features of the inhibition mechanism for this enzyme. In contrast, the *B. cereus* I enzyme was not fully inhibited in the presence of a 16,000-fold excess of clavulanate (52), indicating that hydrolysis of the inhibitor is occurring at a rapid rate.

Sulbactam. Sulbactam was developed as a potential β-lactamase inhibitor that might exhibit some of the same properties as clavulanic acid (55). In initial studies, it was apparent that the two inhibitors had similar inhibitory profiles. Cephalosporinases were inhibited less effectively than were penicillinases or broad-spectrum β-lactamases (Table 5). However, the differential between the enzyme classes was not as great as observed with clavulanic acid in that sulbactam was slightly more active against cephalosporinases than clavulanic acid.

In addition to having similar inhibition profiles, both β-lactams exhibited a time-dependent mode of inhibition. When the mechanism of action of sulbactam was examined, it was apparent that the same kind of inhibitory pathway was operative for both clavulanic acid and sulbactam.

In extensive studies of sulbactam with the *Escherichia coli* TEM-2 β-lactamase, the sulfone was observed to form a transiently inhibited complex with the enzyme; hydrolysis of sulbactam was also observed before irreversible inactivation resulted (21). Some 3,100 molecules of sulbactam were hydrolyzed before complete inactivation of the enzyme occurred (Table 4). Thus, on this basis, clavulanic acid should be at least 25 times more effective than sulbactam in inhibiting the TEM β-lactamases.

Other β-lactam inhibitors. In the clinical setting the only β-lactams used specifically as β-lactamase inhibitors are clavulanic acid and sulbactam. As discussed above, both of these inhibitors act on the same class of β-lactamases, i.e., the common broad-spectrum β-lactamases or the penicillinases. Neither of these compounds is very effective against the inducible chromosomal cephalosporinases that are becoming one of the most serious factors in the development of nosocomial infections (117, 129).

Other β-lactamase inhibitors, including YTR 830, aztreonam, SQ 27,327, and AMPA (Fig. 4), have been tested for activity against these cephalosporinases. YTR 830 is another penicillanic acid sulfone that has been used to protect aminopenicillins. In vitro studies indicated that this inhibitor inactivated the common P99 cephalosporinase from *Enterobacter cloacae* with less than 20 turnovers of inhibitor per molecule of enzyme (K. Bush, unpublished data). Most studies with this inhibitor, however, have been performed in whole cells, and the impressive inactivation data do not translate into synergistic activity with labile penicillins in *Enterobacter* spp. (11, 77).

Aztreonam is the first narrow-spectrum monobactam available for clinical use. Although this molecule was designed to be a potent antibiotic in its own right (137), it is also a potent inhibitor of cephalosporinases, with K_i values in the nanomolar range (37). Aztreonam forms stable covalent acyl enzymes with the *Enterobacter* P99 and E2 β-lactamases, with half-lives of 6.8 (30) and 2.3 (37) h, respectively. Although the end result of these interactions is hydrolysis of

TABLE 4. Inactivation of β-lactamases by irreversible inactivators

β-Lactamase	Molar ratio of inhibitor/enzyme required for inactivation (reference)	
	Clavulanate	Sulbactam
Staphylococcus aureus PC1	1 (40)	ND[a]
Escherichia coli TEM-2	115 (60)	7,000 (59) 3,100 (138)
Bacillus cereus I	>16,000 (52)	ND
Streptomyces albus G	20,000 (64)	ND
Actinomadura sp. strain R39	400 (64)	ND

[a] ND, Not determined.

TABLE 5. Inhibition of β-lactamases by the penicillanic acid sulfone sulbactam and YTR 830

β-Lactamase	Type[a]	K_i (μM)	
		Sulbactam	YTR 830
Escherichia coli	Cephase	66[b]	ND[c]
Proteus vulgaris	Cephase	16[b]	ND
Morganella morganii	Cephase	34[d]	2.3[d]
Serratia marcescens	Cephase	120[d]	229[d]
E. coli TEM-1	Broad spectrum	0.9[b]	ND
Klebsiella pneumoniae K1	Broad spectrum	1.6[b]	ND
E. coli OXA-2	Penase	0.1[b]	ND

[a] See footnote *a*, Table 3.
[b] Reference 91.
[c] ND, Not determined.
[d] Reference 73.

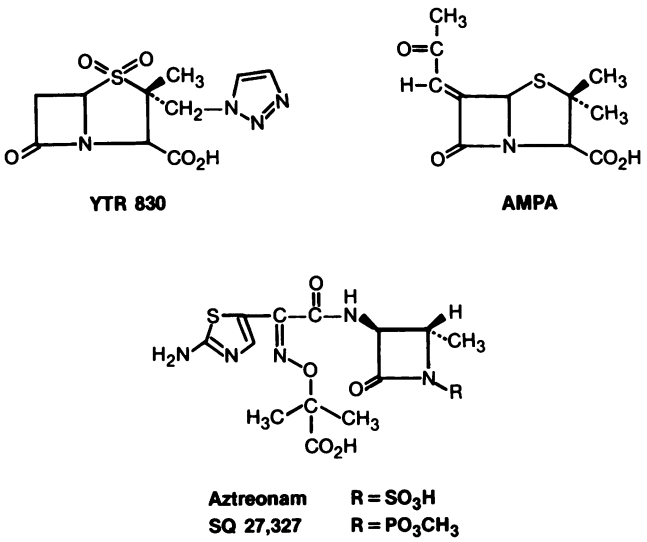

FIG. 4. Structures of potentially useful cephalosporinase inhibitors.

the monobactam and release of active enzyme, the time period is such that the enzyme remains effectively inactivated for several generations of bacterial growth. Similar activity has been observed with cephalosporinases from *Serratia* sp. (32) and *Citrobacter*, *Morganella*, and *Pseudomonas* spp. (Bush, unpublished data). Aztreonam does not bind well to penicillinases or broad-spectrum β-lactamases; therefore, it is ineffective as an inhibitor of these enzymes (30; Bush, unpublished data). Monophosphams (87), another class of monobactams, also interact strongly with the cephalosporinases from *Enterobacter cloacae* (31). SQ 27,327, a typical member of this group, not only has a low K_i for the initial binding of inhibitor to enzyme, but also eventually inactivates the β-lactamase. Like aztreonam, monophosphams interact poorly with penicillinases and broad-spectrum β-lactamases.

Other potent β-lactamase inhibitors include the 6-acylmethylenepenicillanic acids (3). Of these, AMPA has been the most thoroughly described (7, 9). The spectrum of inhibitory activity is broader than that of either clavulanic acid or sulbactam, with higher activity against all enzymes tested than either of these inactivators (9). Although its activity is comparable to that of sulbactam with the *Enterobacter cloacae* cephalosporinases, it is at least 1 order of magnitude better than sulbactam and several orders of magnitude better than clavulanic acid in its inhibition of other cephalosporinases. AMPA interacts with the TEM β-lactamases similarly to clavulanate or sulbactam in that transiently inhibited enzyme exists before complete inactivation is accomplished (8). No more than 50 molecules of inhibitor per molecule of enzyme are required before inactivation is observed, thereby explaining its superior activity (10).

Many other β-lactamase inhibitors have been described in the literature. Among these are the halopenicillanic acids, with a spectrum of activity similar to that of clavulanic acid (85, 118, 144), other penicillanic acid sulfones (59, 101, 146), and carbapenems (20, 74, 109, 111). Non-β-lactam-containing inhibitors have also been reported (see reference 41), but their activities and specificities are not as impressive as those of the molecules discussed above.

IN VITRO ACTIVITY

Antimicrobial Activity of Inhibitors Alone

Because all of the molecules discussed above are β-lactams, there is the possibility that any of these β-lactamase inhibitors may possess antibacterial activity in their own right. Both of the monobactams, aztreonam and SQ 27,327, were designed to be antibiotics, with their enzyme inhibitory activity an unanticipated addition to their antibacterial action. However, the other molecules were evaluated in initial testing primarily as β-lactamase inhibitors.

Clavulanic acid, sulbactam, and AMPA all exhibit affinities for penicillin-binding proteins (PBPs), the enzymes responsible for cell wall synthesis that serve as the killing targets for β-lactam antibiotics (65, 133). Lethal targets in *E. coli* are PBPs 1a and 1b together, PBP 2 alone, or PBP 3 alone. Synergistic effects among the four targets may also result in cell death.

Clavulanate binds most effectively to PBP 2 in *E. coli*, causing a primary morphological response of swollen, bulging cells, followed by cell lysis and the release of spheroplasts (134). In contrast, sulbactam binds most tightly to PBP 1a in *E. coli*, with weaker binding to PBPs 1b and 2 (90). In a similar manner, AMPA binds best to PBP 1a, with weak binding to PBPs 1b, 2, and 3 (7). Thus, these β-lactams should possess some discernible antibiotic activity.

As predicted from the PBP data, both clavulanate and sulbactam are weak antibiotics in their own right. However, AMPA was not active against *S. aureus* or members of the family *Enterobacteriaceae* at concentrations up to 50 μg/ml. From the minimum inhibitory concentration (MIC) data presented in Table 6, it is apparent that clavulanate exhibits weak antibiotic activity against most of the *Enterobacteriaceae* as well as against gram-positive and anaerobic organisms. Moderate antibacterial activity was observed with *Haemophilus influenzae*. However, clavulanate exhibits good activity against most isolates of penicillinase-producing *Neisseria gonorrhoeae*.

Antimicrobial activity of sulbactam (Table 7) is similar to that seen with clavulanate. There is poor activity against the gram-positive organisms and members of the *Enterobacteriaceae*. Like clavulanate, the strongest antibacterial activity is against *N. gonorrhoeae*. However, the antimicrobial activity of sulbactam against *H. influenzae* is weak. Discernible activity against *Bacteroides fragilis* was observed.

TABLE 6. Antimicrobial activity of clavulanic acid alone against selected organisms

Organism	β-Lactamase[a]	MIC (μg/ml)	Reference
Staphylococcus aureus Russell	Penase	15	120
Klebsiella aerogenes NCTC 418	Broad spectrum	31	120
Proteus mirabilis C889	Penase	62–125	120
Escherichia coli JT39	Broad spectrum	31	120
Pseudomonas aeruginosa Dalgleish	Penase	250	120
Neisseria gonorrhoeae	Broad spectrum	1–5	102
		0.1[b]	107
Branhamella catarrhalis	Broad spectrum		
Haemophilus influenzae	Broad spectrum	6.3	107
Bacteroides fragilis	Penase	>50	107

[a] Penase, Penicillinase; broad spectrum, broad-spectrum substrate profile.

[b] MIC for 75% of isolates tested.

TABLE 7. Antimicrobial activity of sulbactam against selected organisms (adapted from reference 55)

Organism	β-Lactamase[a]	MIC (μg/ml)[b]
Staphylococcus aureus	Penase	200
Klebsiella pneumoniae	Broad spectrum	50
Proteus morganii	Penase	100
Escherichia coli	Penase	200
	Cephase	50
Pseudomonas aeruginosa	Cephase	>400
Neisseria gonorrhoeae	Broad spectrum	1.2
Haemophilus influenzae	Broad spectrum	100
Bacteroides fragilis	Cephase	25

[a] Penase, Penicillinase; broad spectrum, broad-spectrum substrate profile; Cephase, cephalosporinase.
[b] MIC determined at an inoculum of 10^6 CFU.

Thus, these molecules not only are potent inhibitors in isolated enzyme studies, but also have the potential for interacting with specific killing targets of growing cells. Although the effects of these β-lactamase inhibitors on PBPs are generally weak, it is possible that the inhibitors may bind to PBPs as a secondary effect after initial interaction with β-lactamases. Thus, the potential for synergy with a labile β-lactam antibiotic has been increased.

Synergistic Activity of Inhibitors

Although the β-lactamase inhibitors described above act as effective inactivators of isolated enzymes, a most important question is whether these molecules can act to protect susceptible β-lactam antibiotics from hydrolysis in growing cells. It is imperative that these inhibitors penetrate the periplasm of gram-negative organisms rapidly so as to intercept the β-lactamase before all labile antibiotic has been destroyed. It is also important that enzyme inhibition occur faster than synthesis of new protein; otherwise, succeeding generations of cells will simply continue to elaborate additional β-lactamase capable of hydrolyzing antibiotic. Therefore, studies in vitro in organisms producing various levels of β-lactamase are essential to show efficacy of a β-lactamase inhibitor in combination with a second β-lactam antibiotic.

Synergy between a susceptible β-lactam antibiotic and a β-lactamase inhibitor can be evaluated by a variety of methods. In some broth dilution studies a single inhibitor concentration is selected, based on the observed inhibitory activity in isolated enzyme studies. MICs of the antibiotic are determined in the presence and absence of this fixed inhibitor concentration, and synergy is evaluated. In other studies equal concentrations of inhibitor and antibiotic are varied stepwise by twofold dilutions, and MICs of the combination are compared with those observed in the absence of inhibitor. Probably the best method used to evaluate synergy is the checkerboard procedure, whereby the largest combinations of antibiotic and inhibitor concentrations can be evaluated. In this procedure synergy is defined as a fourfold reduction in MIC for both components (107).

Standard procedures for disk diffusion susceptibility testing have been outlined for Augmentin (a combination of 2 parts amoxicillin to 1 part clavulanic acid) (67), Timentin (a combination of 15 parts ticarcillin to 2 parts clavulanic acid) (66, 78), cefoperazone-sulbactam (79), and ampicillin-sulbactam (17). However, a cautionary note has been reported, indicating that susceptibility test results may differ according to the medium used. It was observed that more strains were determined to be susceptible to Augmentin when tested on Mueller-Hinton medium versus testing performed on Oxoid diagnostic sensitivity test agar (25).

In broth dilution assays clavulanic acid was shown to be synergistic with a number of penicillins and cephalosporins that are readily hydrolyzed by plasmid-mediated β-lactamases. In addition to the protection of amoxicillin, ampicillin, mezlocillin, cephaloridine, ticarcillin, and piperacillin in penicillinase-producing *S. aureus* (Table 8), the following β-lactam antibiotics have also been reported to be protected by clavulanate: penicillin G (15), cefamandole (107), cephalothin (15), azlocillin (16), cefoperazone (45), furbenicillin (96), and carbenicillin (51). Clavulanic acid failed to show any synergism with cefoxitin in cefoxitin-resistant clinical isolates of *Bacteroides fragilis* (48).

Synergy was demonstrated when clavulanic acid and amoxicillin were tested in a wide variety of β-lactamase-producing bacteria. Excellent protection was afforded amoxicillin when clavulanic acid was added to gram-negative, gram-positive, and anaerobic organisms initially resistant to this labile antibiotic (Table 9). Strains most impressively inhibited included *Bacteroides fragilis*, *Branhamella catarrhalis*, *E. coli*, *Haemophilus* spp., *Enterobacter aerogenes*, *N. gonorrhoeae*, *Proteus* spp., *Citrobacter diversus*, and *S. aureus*. Other organisms that have been reported to be susceptible to clavulanic acid-penicillin combinations include *Enterobacter agglomerans* (114), *Klebsiella oxytoca* (96), *Klebsiella ozaenae* (75), *Mycobacterium tuberculosis* (132), *Providencia alcalifaciens* (75), *Pseudomonas cepacia* (75), *P. maltophilia* (114), *Pseudomonas pseudomallei* (75), *Salmonella anatum* (73), *Salmonella typhimurium* (75), *Serratia liquefaciens* (96), and *Shigella sonnei* (75).

Organisms such as *Enterobacter* spp., *Serratia* spp., and *Citrobacter freundii* that were not responsive to combinations of clavulanic acid and amoxicillin (Table 9) generally produced inducible cephalosporinases, shown previously to be poorly inhibited by clavulanic acid. Many strains of penicillin-resistant *Pseudomonas aeruginosa* also did not respond well to combinations with clavulanic acid (114, 143). However, some of these effects may be due in part to poor penetration of the organisms by the β-lactams used in the study.

Although sulbactam has been studied primarily for synergy with the aminopenicillins ampicillin and amoxicillin, it has also been used to protect cefoperazone (50), cephaloridine (96), carbenicillin (96), and furbenicillin (96). Synergistic activity of sulbactam combined with ampicillin is shown in Table 10. Profiles similar to that observed with clavulanic acid were observed. Synergy was evident at ampicillin-sulbactam concentrations of 16:8.0 μg/ml (or less) in *Acinetobacter calcoaceticus*, *Bacteroides fragilis*, *Branhamella catarrhalis*, *N. gonorrhoeae*, *Citrobacter* spp., *H. influ-*

TABLE 8. Protection of β-lactam antibiotics by clavulanic acid (CA) in β-lactamase-producing *S. aureus* (methicillin susceptible)

Antibiotic	*n*	MIC (μg/ml)		Reference
		β-Lactam	β-Lactam/CA	
Amoxicillin	29	8.0[a]	0.5/8.0[a]	11
Ampicillin	1	500	0.02/5.0	120
Mezlocillin	8	256	4.0/1.0	145
Cephaloridine	1	0.6	0.06/5.0	120
Ticarcillin	1	200	25/1.7	82
	1	128	8.0/5.0	72
Piperacillin	1	>200	1.6/0.4	108

[a] MIC for 90% inhibition.

TABLE 9. Protection of amoxicillin (AMX) by clavulanic acid (CA) in β-lactamase-producing organisms

Organism	n	MIC_{90} (μg/ml)[a]		Reference
		AMX	AMX/CA	
Bacteroides fragilis	28	33[b]	0.48/1.0[b]	75
Branhamella catarrhalis	35	2.0	0.125/0.062	4
	53	8.0	0.25/ND[c]	5
Citrobacter diversus	8	128	2.0/8.0	11
Citrobacter freundii	12	>128	>128/8.0	11
Escherichia coli (R^+)	100	>5,000[b]	3.0/10[b]	75
	21	>128	8.0/8.0	11
Enterobacter spp.	25	>128	>128/8.0	11
Haemophilus spp.	132	>32	2.0/1.0	92
	15	150[b]	1.1/0.5[b]	75
Enterobacter aerogenes	45	315[b]	1.75/1.0[b]	75
Mycobacterium tuberculosis	13	>32	4.0/4.0	132
Neisseria gonorrhoeae	6	>40[b]	0.44/0.5[b]	75
Proteus spp.	23	433[b]	5.0/2.5[b]	75
Serratia spp.	20	>128	>128/8.0	11
Staphylococcus aureus	35	197[b]	1.1/0.5[b]	75

[a] MIC for 90% inhibition.
[b] Geometric mean MIC.
[c] ND, No data.

enzae, *K. pneumoniae*, *M. tuberculosis*, and both methicillin-susceptible and methicillin-resistant *S. aureus*. Although the synergistic activity with sulbactam in general was equivalent to or less than with clavulanic acid, improved activity over clavulanate was observed with some *Citrobacter* strains.

As might be predicted from the isolated enzyme studies, synergy in β-lactamase-producing *E. coli* strains was much greater with clavulanic acid than with sulbactam. This would be expected, if one assumes that the majority of these strains produce plasmid-mediated, TEM-type, broad-spectrum β-lactamases. When a plasmid is present in a high copy number, the elevated level of β-lactamase produced will hydrolyze sulbactam more quickly than the enzyme will become inactivated because of the reasonably high turnover number (Table 4). This was demonstrated in studies by Easton and Knowles (53), who observed good synergy with both sulbactam and clavulanic acid in a *Proteus* species that produced a moderate level of TEM β-lactamase. However, in an *E. coli* strain that produced 40 times more TEM enzyme, only a marginal enhancement of activity was determined for an ampicillin-sulbactam combination, whereas a 200-fold enhancement was observed with the ampicillin-clavulanate duet. Thus, the amount of enzyme produced strongly affects the observed level of synergy for these inhibitors.

Another factor that will contribute to a differential in activity is the ease with which the inhibitor can penetrate the cell. As indicated by Li et al. (96), clavulanic acid may have less of a penetration problem than sulbactam in *P. aeruginosa*, *Citrobacter*, and *Enterobacter* strains.

In an attempt to expand the use of β-lactamase inhibitors, synergy studies with YTR 830 have also been performed as a means to protect amoxicillin (11) or the extended-spectrum penicillins ticarcillin, piperacillin, mezlocillin, and apalcillin (77). In general, YTR 830 exhibited better synergy than sulbactam and was comparable to clavulanic acid in the inhibition of *S. aureus* and gram-negative organisms. Among pseudomonads, YTR 830 exhibited a decided synergistic advantage only with *P. cepacia* (77).

Although most studies in vitro indicated that β-lactamase inhibitors acted in synergy with or were indifferent to the presence of an added penicillin or cephalosporin, antagonism of ticarcillin by increasing concentrations of clavulanic acid has been reported in members of the *Enterobacteriaceae* (141). A clinical isolate of *Enterobacter cloacae* susceptible to ampicillin but resistant to Augmentin has also been reported (47). One explanation for this behavior is induction of cephalosporinases by the second β-lactam. Induction of β-lactamase activity in these organisms can have serious consequences in a clinical setting (117, 128, 129). It is especially important to know whether the β-lactamase inhibitors act as inducers of the cephalosporinases, because this group of enzymes is not well inhibited by

TABLE 10. Protection of ampicillin (AMP) by sulbactam (SUL) in β-lactamase-producing organisms

Organism	n	MIC_{90} (μg/ml)[a]		Reference
		AMP	AMP/SUL	
Acinetobacter calcoaceticus	20	50	3.1/3.1	122
Bacteroides fragilis	26	25	3.2/1.6	122
	70	>256	8.0/4.0	142
Branhamella catarrhalis	15	12.5	0.4/0.4	122
Citrobacter diversus	8	32	2.0/8.0	11
Citrobacter freundii	12	128	16/8.0	11
Escherichia coli (R^+)	21	>128	>128/8.0	11
	150	>128	16/16	122
Enterobacter spp.	25	>128	>128/8.0	11
Enterobacter aerogenes	14	128	16/16	122
Enterobacter cloacae	28	>128	>128/>128	122
Haemophilus influenzae	14	64	<0.25/8.0	11
	20	>64	2.0/1.0	122
Klebsiella oxytoca	18	>128	16/16	122
Klebsiella pneumoniae	42	>128	8.0/8.0	122
Mycobacterium tuberculosis	13	>32	8.0/8.0	132
Neisseria gonorrhoeae	12	10[b]	2.5/2.5[b]	122
Proteus spp.	23	>128	8.0/8.0	122
Proteus, Providencia, Morganella spp.	25	>128	>128/8.0	11
Serratia spp.	20	>128	>128/8.0	11
	21	>128	32/32	122
Staphylococcus aureus				
Methicillin susceptible	29	8.0	0.5/8.0	11
	70	128	2.0/2.0	122
Methicillin resistant	75	>128	16/16	122

[a] MIC for 90% inhibition.
[b] MIC for 100% of strains tested.

clavulanate or sulbactam. As indicated above, increased amounts of enzyme due to induction may result in therapeutic failure when complex enzyme kinetic interactions are involved.

Several studies have evaluated the induction potential of β-lactamase inhibitors. In strains of *Enterobacter cloacae* (90, 103), *P. aeruginosa* (90), and *Proteus rettgeri* GN4430 (147), clavulanic acid was a good inducer of cephalosporinase activity. In selected *Proteus vulgaris* strains (90, 112, 147), poor induction of β-lactamase activity was reported for clavulanate, although in one strain sulbactam was a good inducer of enzyme activity (147). In *Morganella morganii* clavulanic acid acted as a weak inducer, whereas sulbactam and YTR 830 did not induce any β-lactamase activity (104). In all of these organisms, ampicillin and cefoxitin were more potent inducers than any of the β-lactamase inhibitors. Although the induction potential of these inhibitors is quite variable depending on the organisms in question, a major clinical problem due to β-lactamase induction does not seem likely.

CLINICAL USAGE

After evaluation of all of the basic in vitro studies of the various β-lactamase inhibitors, the real question remaining is whether these molecules will be effective in protecting labile β-lactam antibiotics used to treat infections. Efficacy of β-lactamase inhibitors in vivo is dependent upon a number of additional parameters. Pharmacokinetic considerations are of utmost importance. Stability in body fluids, tissue penetration, metabolism, renal clearance, and, most importantly, safety must also be evaluated. Because these inhibitors are combined with some of the best-studied and safest antibiotics in existence, it is absolutely necessary that any drug combinations containing older penicillins or cephalosporins be just as innocuous.

Clavulanic Acid

Augmentin: amoxicillin-clavulanic acid. Augmentin, a 2:1 combination of amoxicillin-clavulanic acid, was the first β-lactamase inhibitor approved for use worldwide. The combination of amoxicillin with clavulanate was selected because the pharmacokinetics of the two components were quite similar. In two studies the serum half-lives ranged from 53 to 75 min for amoxicillin and 47 to 60 min for clavulanic acid (2, 14). In a study in normal human volunteers, using a therapeutic combination containing 500 mg of amoxicillin and 125 mg of clavulanic acid, serum half-lives for each component were not significantly different from the values obtained for each component tested separately; peak serum levels were 8.0 mg/liter for amoxicillin and 3.9 mg/liter for clavulanate given in combination (2). However, the renal clearance of clavulanic acid, a moderately unstable moiety at physiological pH, was increased significantly in the presence of amoxicillin. Excretion of amoxicillin was independent of clavulanate. No other differences in pharmacokinetics were observed when the individual parameters for amoxicillin and clavulanate were compared with the values obtained with the combination therapy.

Augmentin has been used to treat a variety of infections, many of which would be expected to be refractory to treatment with amoxicillin alone. Outpatient treatment is the area of greatest impact of Augmentin, as this is the area in which orally absorbed penicillins are most used.

Augmentin is efficacious in treating respiratory infections (22, 81), skin and soft-tissue infections (22), bacteriuria in pregnant women (116), chancroid (105), penicillinase-producing *N. gonorrhoeae* (83, 93, 113), and acute otitis media in pediatric patients (110). Augmentin has perhaps been most studied in treating urinary tract infection (UTI) (28, 44, 68, 95). Although it has been used successfully in the treatment of complicated and uncomplicated, recurrent, and nosocomial UTI as well as bacteriuria, the optimal dosages and length of treatment are details that need to be evaluated further. In one study, the proportion of fecal *E. coli* resistant to amoxicillin alone was increased from 13 to 39% after a 1-week treatment of UTI by Augmentin (76).

Side effects have included mild gastrointestinal disturbances such as nausea, vomiting, and diarrhea (22). A higher incidence of side effects occurred with a higher dosage regimen of clavulanate (44). When Augmentin is taken with food, the incidence of side effects is reduced (135).

Timentin: ticarcillin-clavulanic acid. Ticarcillin is an injectable broad-spectrum penicillin that has reasonable stability to hydrolysis by cephalosporinases but which can be hydrolyzed by certain plasmid-mediated penicillinases (91). The combination of ticarcillin and clavulanic acid has been approved for use in the United States as an injectable antibiotic combination that is often administered with an aminoglycoside in compromised patients (26, 49, 98). Timentin is available as a combination of 3 or 5 g of ticarcillin plus 200 or 100 mg of clavulanate.

Pharmacokinetic analyses for the two components administered at a 15:1 ratio of ticarcillin/clavulanic acid showed that the amount of clavulanate in both serum and blister fluid continually decreased relative to the ticarcillin concentration; after 4 h the ratios were 40:1 in serum and 55:1 in blister fluid (18). Although serum levels were dramatically reduced with time, clavulanate concentrations appeared to be high enough to maintain enzyme inhibition.

Therapeutic use of this combination results in coverage of most members of the *Enterobacteriaceae*, *H. influenzae*, *Bacteroides fragilis*, *P. aeruginosa*, and pencillinase-producing *S. aureus* (43, 125). Timentin has been used to treat lower respiratory infections (58), febrile neutropenic patients (26), UTI (43), pneumonia (126), and osteomyelitis (126). Clinical cures in treatment of female pelvic infection were comparable to those obtained with cefoxitin (115). Likewise, the two regimens were judged to be similar in the prophylaxis of infection after Caesarean section (127).

Side effects reported in clinical trials appeared to be due primarily to ticarcillin, the predominant component in the combination (140). Problems reported include gastrointestinal disturbances (46) and impairment of platelet function (46, 140). Overall, side effects were mild and did not cause discontinuance of therapy.

Sulbactam

Sultamicillin. Sultamicillin is an orally absorbed double-ester prodrug of ampicillin and sulbactam. In mammals, equimolar amounts of ampicillin and sulbactam are formed rapidly, probably due to hydrolysis by esterases in the intestinal epithelium (13, 54). This covalent combination was particularly attractive because both compounds could be formed quantitatively with >80% oral absorption in the human intestine (125).

In rats, sultamicillin yielded 2 to 2.5 times greater bioavailability for ampicillin and sulbactam than when each was administered separately. Distribution of the two components in rats indicated that both were present in equal amounts in plasma, but distributed differently in various tissues. More

equal distribution of the two components resulted when ampicillin and sulbactam were administered as sultamicillin, compared with administration of the two β-lactams individually (54). In humans, when normal males were given a single 750-mg oral dose of sultamicillin, the mean half-lives for sulbactam and ampicillin were 1.11 and 0.96 h, and peak plasma concentrations were 8.9 and 9.1 mg/liter, respectively. Urinary excretion was 66% for sulbactam and 59% for ampicillin (125).

Sultamicillin was shown to be effective in the treatment of UTI (13), acute sinusitis (80), lower respiratory tract infections caused by *H. influenzae* (119), pediatric streptococcal pharyngitis (12), uncomplicated gonorrhea (57), superficial skin and soft-tissue infections (71), and otitis media (70). However, gastrointestinal side effects, especially diarrhea, were reported in most studies (12, 57, 80, 119, 125). This may be due to selective pressure on intestinal flora caused by residual ampicillin, protected from hydrolysis by unabsorbed sulbactam.

Ampicillin-sulbactam. When administered orally, sulbactam is not well absorbed (62). Therefore, if this penicillanic acid is to be used therapeutically, it must be coadministered parenterally with a second broad-spectrum β-lactam antibiotic. Although sulbactam has been used with both the cephalosporin cefoperazone and the aminopenicillins, the most common use of this inhibitor is in combination with ampicillin.

Ampicillin is an excellent partner for sulbactam when the pharmacokinetics of the two are considered. When given in a single dose (500 mg of each), neither component has any effect upon the kinetic parameters of the other (61). When a dosage regimen of 2 g of ampicillin and 1 g of sulbactam was given, peak serum concentrations of >94 μg of ampicillin and 41 μg of sulbactam were measured, with half-lives for the two drugs of 1.0 to 1.1 h. The principal route of excretion was via the urine, with recoveries of >75% for both β-lactams (63).

Therapeutic applications for the ampicillin-sulbactam combination include gynecological infections caused by anaerobes only or by mixed populations of aerobes and anaerobes (69), polymicrobial intraabdominal infections (136), soft-tissue, bone, and joint infections (97), and penicillinase-producing *N. gonorrhoeae* (84). Sulbactam alone was not judged sufficiently suitable for the treatment of uncomplicated gonorrhea (38). Because both sulbactam and ampicillin can penetrate the cerebral spinal fluid, the combination has been used successfully to treat pediatric bacterial meningitis (124). This combination has also been used prophylactically in surgical procedures and appears to have efficacy similar to that of alternative antibiotic regimens (39). An ampicillin-sulbactam regimen has been favorably compared with treatment with cefoxitin in acute salpingitis (27) and other obstetric and gynecological infections (131).

The ampicillin-sulbactam combination has been remarkably free of major side effects. The most consistent complaint has been pain at the injection site (94).

FUTURE PROSPECTS

Desirable Properties for New Inhibitors

Sulbactam and clavulanic acid have given clinicians an opportunity to use well-tolerated and efficacious aminopenicillins for the treatment of many infections caused by β-lactamase-producing bacteria. However, the lack of inhibitory activity of these new agents against cephalosporinases poses limitations in the use of these molecules. Thus, additional classes of inhibitors could be useful.

Any new β-lactamase inhibitors should have the following characteristics. The molecules must be capable of preventing hydrolysis of a well-tolerated broad-spectrum β-lactam antibiotic, preferably an inexpensive penicillin. The pharmacokinetics of the two molecules should be similar. Side effects should be minimal and mild. Ideally, one would like to have molecules with oral activity. The molecules should not be good inducers of cephalosporinase activity.

Potent inactivation of cephalosporinase activity would be an attractive addition to the spectrum of activities available with clavulanic acid or sulbactam. It is interesting to note that YTR 830 acts as a good inactivator in isolated enzyme studies with the *Enterobacter* cephalosporinases, yet synergy with common β-lactam antibiotics is not observed in the organisms producing these enzymes. Therefore, good inactivation by itself is not sufficient for good activity.

It is important that these inhibitors meet the following criteria regarding the plasmid-mediated TEM-type broad-spectrum β-lactamases, the most common β-lactamases among the members of the *Enterobacteriaceae* (36). The inhibitor must inactivate the TEM-type enzymes at low inhibitor concentrations, or it must be totally inert to hydrolysis by these enzymes. If a cephalosporinase inhibitor does not interact with the TEM enzymes, the inhibitor would have to be combined with a β-lactam antibiotic that exhibits excellent stability to these broad-spectrum β-lactamases.

Limitations of Combination Therapy

Combination therapy, using antibiotics from any of a number of classes, has been used to expand the spectrum of activity for many narrow-spectrum antibiotics. In addition to the possibility of cost advantages when older, relatively inexpensive antibiotics are used, combination therapy is especially advantageous in the treatment of neutropenic or otherwise compromised individuals for whom broad-spectrum coverage is necessary immediately. In certain instances, a double β-lactam combination can offer a safer form of therapy than a β-lactam–aminoglycoside combination.

However, certain problems arise when combination therapies are used. Especially in oral formulations, a fixed ratio of antibiotics is available. This results in at least two major problems: tissue distribution may be unequal and different dosages might provide better coverage at multiple sites; identification of the organism to be treated may indicate that a different ratio of inhibitor/antibiotic would provide better therapy.

The greatest risk involved in combination therapy with two β-lactams is the higher probability of selecting resistant mutants. Resistance can result from induction of β-lactamases that can hydrolyze the active antibiotic or that are increased in concentration such that the inhibitor is no longer effective. Permeability mutants can arise as a result of selective pressure from either β-lactam. Also, possible antagonism could result from competition for entry through the porins (73).

Alternatives to β-Lactamase Inhibitors

Many pharmaceutical companies have considered a variety of solutions to the resistance problems caused by β-lactamases. One alternative is to substitute a different class of antibiotic such as the quinolones for the β-lactamase-

labile aminopenicillins. Another approach is to develop β-lactamase-stable β-lactams. Among this group are the carbapenems such as imipenem, the cephamycins such as cefoxitin, the oral cephalosporins such as cephradine, the stable penicillins such as cloxacillin, and the monobactams such as aztreonam.

The advantages of these molecules is that a single molecular entity is available for interaction with the bacterial cell. Philosophical arguments abound as to whether these should be antibiotics with a narrow spectrum of activity (e.g., aztreonam), thereby minimizing the possibility of adverse effects due to opportunistic infections, or extraordinarily broad-spectrum antibiotics (e.g., imipenem) that destroy virtually every bacterium in the vicinity.

CONCLUSIONS

Therapeutic control of β-lactamase-producing bacteria has been a major clinical problem for at least 40 years. Development of drug combinations containing the β-lactamase inhibitors clavulanic acid and sulbactam has given clinicians a novel approach to controlling resistant organisms.

On the basis of inhibition studies with isolated enzymes, clavulanic acid is a better inhibitor of the broad-spectrum β-lactamases than sulbactam. Neither inhibitor is effective with cephalosporinases. In general, the microbiological spectrum of activity of the two inhibitors combined with an aminopenicillin is similar; however, clavulanic acid is more effective when high enzyme producers are encountered. Because the sulbactam-ampicillin combination is given parenterally, higher blood levels may be attained compared with the orally absorbed clavulanate-amoxicillin formulation. These combinations are well tolerated and represent the opportunity for continued use of the safe and well-established aminopenicillins in infections caused by both aerobic and anaerobic bacteria. However, the question remains as to whether these drugs represent real therapeutic advantages compared with some of the β-lactamase-stable antibiotics currently available.

A major problem with the use of the β-lactam combinations is the potential for resistance development. To minimize the development of resistance, some simple advice has been offered by H. Neu (106): treat only infections, not colonizations, and use only a single antibiotic when possible at the lowest appropriate dosage. When high levels of β-lactams are used, resistance to these entities may be observed as a result of permeability changes. However, the major causes of resistance are still related to β-lactamase production. Aminopenicillins such as ampicillin and amoxicillin are good inducers of many chromosomally mediated β-lactamases. In isolated strains clavulanate also has the ability to induce cephalosporinase activity, resulting in enzymes not readily inhibited by clavulanate. Should these β-lactam antibiotic combinations be used indiscriminately, the development of gram-negative superinfections may result.

Thus, it appears that β-lactamase inhibitor combinations can be useful, but only when warranted. Although both clavulanic acid and sulbactam have been used successfully in a variety of clinical situations, they should be prescribed only if a susceptible β-lactamase-producing organism is the causative agent. Combination therapy should not be used when a single agent would be effective. However, cost considerations may make amoxicillin-clavulanic acid combinations attractive compared with cephalosporins such as cefaclor (68). Ideally, one would like an antibiotic that is efficacious, specific, inexpensive, noninducing, and exquisitely stable to hydrolysis by common β-lactamases. Until this antibiotic is developed, combination therapy with β-lactamase inhibitors appears to fill a useful niche in the treatment of bacterial infections.

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