Penicillins and β-lactamase inhibitor combinations

Katrina L. Mealey, DVM, PhD, DACVIM, DACVCP

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Comments and manuscripts should be sent to Cyril R. Clarke, BVSc, PhD, DACVCP, Department of Anatomy, Pathology and Pharmacology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, Telephone 405/744-8093.

The β-lactam family of antibiotics (penicillins, cephalosporins, monobactams, and carbapenems) are widely used in veterinary medicine because of their spectrum of activity, favorable safety profile, and reliable clinical efficacy. Widespread use of these agents has resulted in emergence of resistant organisms.[1] Gram-positive and gram-negative bacteria can become resistant to β-lactam antibiotics via production of β-lactamase, an enzyme that irreversibly alters the chemical structure of the antibiotic, abolishing its antibacterial activity.[2] Production of these enzymes has vitiated the utility of benzylpenicillin against staphylococci and has greatly undermined that of ampicillin against enterobacteria.[1] Several classes of β-lactamase enzymes exist, differing in the species of bacteria that produce them, whether they are plasmid or chromosomal in origin, whether they are constitutively expressed or inducible, their mechanism of action, and the spectrum of β-lactam antibiotics they are capable of hydrolyzing. The combination of a β-lactamase inhibitor with a traditional β-lactam antibiotic offers stability against the inactivating β-lactamase and an expanded spectrum of activity of the primary antibiotic. Three β-lactamase inhibitors have been approved for use by the FDA: clavulanate (approved for veterinary use), sulbactam, and tazobactam. Clavulanate is commercially available in combination with amoxicillin or ticarcillin, sulbactam is available in combination with ampicillin, and tazobactam is available in a formulation with piperacillin.[3] Substantial pharmacokinetic differences exist between the 3 inhibitors, and these differences should be considered when selecting combinations of a β-lactam antibiotic and β-lactam inhibitor. The currently available β-lactamase inhibitors are primarily active against only 1 class of β-lactamase enzyme.[4]

From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA 99164-6610.

β-Lactamases

β-Lactamases (also known as penicillinases or cephalosporinases) are plasmid or chromosomally encoded bacterial enzymes that catalyze hydrolysis of the amide bond of the β-lactam ring, producing acidic derivatives with no antibacterial activity.[1,2] Production of β-lactamases is the most common mechanism of bacterial resistance to penicillins and cephalosporins.[5] However, fundamental differences exist among the mechanisms for gram-positive and gram-negative bacteria to develop β-lactamase-mediated resistance.

Gram-positive bacteria produce a large amount of β-lactamase (up to 1% of the dry weight of the bacterium) that is secreted extracellularly into the environment.[1,2] These enzymes are generally encoded by plasmids, may be inducible, and may be transferred to other bacteria.[6] β-Lactamases produced by gram-negative bacteria may be chromosomal or plasmid-borne, and their expression may be constitutive (enzyme synthesized constantly at a fixed concentration) or inducible (only trace amounts of β-lactamase enzymes are produced in the absence of β-lactam antibiotic, but transient high production occurs while antibiotic is present).[7] Although gram-negative bacteria have inducible forms of β-lactamase genes, they generally produce smaller quantities of β-lactamase enzyme than do gram-positive bacteria but secrete the enzyme into a strategic location within the periplasmic space.[1,2] The periplasmic space, located between the inner and outer cell membranes, is the site of action for β-lactam antibiotics. A relatively small amount of β-lactamase secreted within the periplasmic space can generate a high enough concentration of enzyme to overwhelm susceptible antibiotics. As of 1998, over 200 distinct β-lactamase enzymes have been isolated.[4] On the basis of their amino acid sequence, β-lactamases have been divided into four molecular classes (A, B, C, and D; **Appendix**). Class-A enzymes are the most commonly found among human clinical isolates and are produced by gram-positive and gram-negative bacteria.[2]

Although β-lactamases existed in bacteria prior to the antibiotic era, clinical use of β-lactam antibiotics is

the major selective factor influencing β-lactamase production by bacteria.[8] In the face of continuing selective pressure, 2 distinct mechanisms have emerged that allow bacteria that produce β-lactamase enzymes to protect themselves from β-lactam antibiotics. One mechanism involves mutations in the β-lactamase gene, encoding enzymes with altered structure and function. Some of these enzymes, called **extended spectrum β-lactamases (ESBL)**, can catalyze hydrolysis of most cephalosporins[9] and are resistant to inactivation by β-lactamase inhibitors.[5] The ESBL are often detected from clinical microbiologic specimens by screening with ceftazidime, because ESBL are routinely resistant to this drug. When such organisms are identified, resistance to all cephalosporins is reported regardless of the actual susceptibilities.[2] More recently, extended spectrum secondary β-lactamases that can catalyze hydrolysis of newer β-lactam antibiotics including carbapenems have been identified in rare human isolates.[2] Most resistance to β-lactam/β-lactamase inhibitor combinations is caused not by these new mutant enzymes but by inducible β-lactamase genes.[10] In the absence of a β-lactam drug, these genes encode only small quantities of enzyme. Once the bacteria are exposed to a β-lactam drug (including β-lactamase inhibitors), synthesis of large quantities of enzyme ensues. Although the individual enzymes are susceptible to the β-lactamase inhibitors combined with the β-lactam drug, they are in such large quantities that the β-lactamase inhibitor is overwhelmed. Any β-lactam drug can induce β-lactamase synthesis; however, some drugs have higher inducer activity than others.[11] For example, clavulanate is a stronger inducer than is ticarcillin, which can be hydrolyzed by the induced enzyme. If bacteria that possess inducible β-lactamase genes are treated with the ticarcillin-clavulanate combination, large quantities of β-lactamase enzyme are subsequently produced. These induced enzymes hydrolyze ticarcillin; therefore, anticipated antimicrobial activity is lost. Conversely, if unprotected ticarcillin is used, less enzyme induction will occur, and the antimicrobial is more likely to be effective.

β-Lactamase Inhibitors

In the 1950s, researchers described the ability of cephalosporin C to inhibit a β-lactamase enzyme produced by *Bacillus cereus*.[4] To identify potent inhibitors of β-lactamase, systematic searches from fermentation broths were undertaken, and the first clinically useful β-lactamase inhibitor, clavulanate, was identified. Although a large number of β-lactamase inhibitor compounds have been synthesized, clinical utility of many of these agents was limited by their pharmacokinetic profiles. Currently, only 2 additional β-lactamase inhibitors, sulbactam and tazobactam, are commercially available. Although these agents differ slightly in structure, their mechanism of action is identical. Each of these agents contains a β-lactam ring that binds to the active site of β-lactamase enzymes with higher affinity than do β-lactam antibiotics.[4, 5,12] This interaction causes the drug and the enzyme to be incapable of further action; therefore, these agents are irreversible inhibitors of β-lactamase and are considered suicide substrates. The reaction between the β-lactamase enzyme and the inhibitor depends on affinity of the receptor sites, pH, and concentration. Generally speaking, tazobactam is the most active of the available β-lactamase inhibitors, and sulbactam is the least active.[13]

Clavulanate

Clavulanate is a β-lactamase inhibitor produced by fermentation of *Streptomyces clavuligerus*. The drug has weak antibacterial activity when used alone but in combination with certain penicillins and cephalosporins shows a synergistic effect that expands the spectrum of activity of the β-lactam antibiotic. Clavulanate is the only β-lactamase inhibitor that can be administered per os and is commercially available in combination with amoxicillin at a 1:4 ratio.[a] Amoxicillin-clavulanate is FDA-approved for use in dogs and cats, and listed indications for its use include skin, soft tissue, and periodontal infections in dogs and skin, soft tissue, and urinary tract infections in cats.[a] This preparation absorbs moisture from the air and may rapidly become inactivated if it is not protected (the commercial preparation is supplied in individually wrapped foil packages). Clavulanate in combination with ticarcillin is commercially available as a parenteral formulation (licensed only for use in human patients).[b]

Limited information is available regarding the pharmacokinetic profile of clavulanate in dogs, and even less data exist for other veterinary species. The pharmacokinetics of amoxicillin appear to be unchanged in the presence of clavulanate.[14] Clavulanate is stable in the presence of gastric acid, and its absorption does not appear to be affected by the presence of food in the stomach. Absorption of clavulanate from the gastrointestinal tract is rapid, and the oral bioavailability in dogs is greater than 30%.[15] The volume of distribution of clavulanate is 0.32 L/kg in dogs.[16] Elimination of clavulanate is by hepatic metabolism and renal excretion of the parent drug and its metabolites.[15] Because the amoxicillin-clavulanate combination is effective in treating experimental *Escherichia coli* cystitis, it is presumed that sufficient concentrations of active drug are excreted in urine.[17,18] Recommended dosages for amoxicillin-clavulanate (combined quantities of both drugs) range from 11 to 22 mg/kg (5 to 10 mg/lb) of body weight PO every 8 to 12 hours.[19,a] Bolton et al[15] found serum concentrations of clavulanate fell more rapidly than did those of amoxicillin after an oral dose of the combination was administered to dogs. The ratio of amoxicillin to clavulanate concentrations in serum was approximately 5:1 at 1 hour, 10:1 at 2 hours, and 20:1 at 3 hours after oral administration of the combination product.[20] Because of the apparently short elimination half-life of clavulanate in dogs, an 8-hour dose interval seems prudent. To the author's knowledge, no data regarding the pharmacokinetics of clavulanate (alone or in combination with amoxicillin) in cats is available.

Clavulanate appears to have a wide margin of safety in most species. The major adverse effects of the amoxicillin-clavulanate combination are related to the gastrointestinal tract and include vomiting and diarrhea.[13,16] In the author's experience, these adverse

effects seem to be more frequent when using the combination product than when using amoxicillin alone. Animals receiving amoxicillin-clavulanate may have false-positive results for glucosuria on some urine dipstick tests. Finally, as with any β-lactam drug, there is a risk of allergic or anaphylactic reactions.[13, 16]

For microbiologic testing of amoxicillin-clavulanate against bacterial isolates, the manufacturer recommends using the Kirby-Bauer amoxicillin-clavulanate susceptibility disk used for testing human isolates.[a] Because the amoxicillin:clavulanate ratio in the human product is 2:1 (the ratio in the veterinary product is 4:1), some question the clinical utility of this in vitro information. However, recent guidelines for amoxicillin-clavulanate and ticarcillin-clavulanate have been published for veterinary pathogens when interpreting disk zone diameters and for determining the breakpoint when using dilution methods.[21]

A 30:1 combination of ticarcillin and clavulanate,[c] approved for use in people, is commercially available as a parenteral formulation.[3] This combination has been used successfully in dogs for treatment of soft-tissue infections.[22] However, little information exists regarding the pharmacokinetic profile of this combination in veterinary species. When identifying a β-lactamase inhibitor to pair with a β-lactam antibiotic, it is important to ensure that the pharmacokinetic properties of drug and inhibitor are similar.[14] Although the pharmacokinetic features of ticarcillin and clavulanate are similar in people, dogs eliminate clavulanate more rapidly than ticarcillin (biological half-lives 39 and 73 minutes, respectively).[23] What effect this may have on antimicrobial efficacy in dogs is not known. It seems prudent to consider that the combination of ticarcillin and clavulanate may be less effective in treating bacterial infections involving β-lactamase-producing organisms in dogs than it is in people. For some bacteria, including *Pseudomonas aeruginosa*, the combination of ticarcillin and clavulanate may not offer an advantage over ticarcillin alone since the β-lactamase produced by this organism is often resistant to clavulanate. Furthermore, since clavulanate may induce bacterial production of β-lactamase, the combination may be less effective for some bacterial infections, particularly *Enterobacter* spp, than ticarcillin alone.

Sulbactam

Like clavulanate, sulbactam is a β-lactamase inhibitor with minimal intrinsic antibacterial activity but, if used in combination with traditional β-lactam antibiotics, will extend its spectrum by irreversibly inhibiting many β-lactamase enzymes. Sulbactam appears to be rapidly but incompletely absorbed in dogs, reaching peak serum concentrations 1 hour after oral administration.[24,25] The oral bioavailability of sulbactam is 19% when administered as a single agent and 49% when administered in combination with ampicillin.[24] Sulbactam is commercially available as a human-approved parenteral product combined with ampicillin in a 2:1 (ampicillin:sulbactam) ratio.[c] The elimination half-lives of sulbactam (0.76 hours) and ampicillin (0.98 hours) are rapid in dogs.[24] Because of the rapid elimination of sulbactam, frequent dosages are necessary to maintain β-lactamase inhibition. Sulbactam extends the bacterial spectrum of ampicillin to include *Staphylococcus, Bacteroides,* and most *Escherichia* organisms but is not effective for treating *Pseudomonas* spp. Because sulbactam is not as potent an inhibitor of β-lactamase enzymes as clavulanate, especially against those often produced by staphylococci and *Bacteroides fragilis*, a higher dose of sulbactam may be necessary. The recommended dosage of ampicillin-sulbactam (combined weight of both drugs) for dogs and cats is 50 mg/kg (22.7 mg/lb) IV every 6 to 8 hours, administered as a slow bolus or as an infusion diluted in saline (0.9% NaCl) solution.[3] Adverse effects are similar to those with clavulanate.

Tazobactam

Tazobactam is also an irreversible inhibitor of β-lactamase enzymes that has minimal intrinsic antibacterial activity. Pharmacokinetic data regarding tazobactam are limited in veterinary species. Renal excretion is the main route of elimination for tazobactam. Interestingly, piperacillin inhibits the renal excretion of tazobactam in dogs in a manner similar to probenecid.[26] This phenomenon may make it less useful than other β-lactamase inhibitor combinations for urinary tract infections. A human-approved product[d] is available in an 8:1 (piperacillin:tazobactam) ratio for parenteral administration. This product provides good activity against many gram-positive, gram-negative (including *Pseudomonas* spp), and anaerobic organisms that are β-lactamase producers. One source recommends a dose (combined weight of both drugs) of 3.4 g/dog every 6 hours to 4.5 g/dog every 8 hours.[3] The reader may wish to consult other references for an appropriate dose for small dogs. Adverse effects are similar to those seen with clavulanate.

Summary

β-Lactamase production by bacteria continues to be one of the main mechanisms of bacterial resistance to β-lactam antibiotics, and it seems likely to remain so. β-Lactamase inhibitors provide 1 strategy to overcome this mechanism of bacterial resistance. Although 3 β-lactamase inhibitor/antibiotic combinations are currently available, only 1 is approved for veterinary use. Because the β-lactamase inhibitor must be present concurrently with the antibiotic for synergistic activity, it is important to consider the pharmacokinetic profile of these drugs in combination. These combinations were developed and optimized for human patients, so it is unlikely that they would achieve the ideal plasma and tissue concentrations and ratios in veterinary patients. Indeed, several differences in pharmacokinetic variables of β-lactam antibiotic/β-lactamase inhibitor agents have been described in dogs, compared with people. Such pharmacokinetic differences should be considered when interpreting in vitro susceptibility results in veterinary species, because these tests use ratios of drug that were established for humans. The β-lactamase inhibitors represent a successful example of targeted drug development. However, the currently available inhibitors are active primarily against class-A β-lactamases. Because the frequency with which class-C

β-lactamases are recognized is rapidly increasing in human isolates, and because β-lactamase enzymes continue to evolve, new β-lactamase inhibitors will need to be developed to target these enzymes.

[a]Clavamox, Pfizer Animal Health, Groton, Conn.
[b]Timentin, SK Beecham, New York, NY.
[c]UnaSyn, Pfizer, New York, NY.
[d]Zosyn, Lederle, Pearl River, NY.

Appendix

Molecular class	Examples	Inhibition by clavulanate	References
A	*Klebsiella* sp, *Proteus vulgaris, Bacteroides* sp (C); *E coli, Staphylococcus* sp (P)	++	5,12
B	*	-	2,4
C	*Pseudomonas aeruginosa, Enterobacter* sp, *Serratia* sp (C)	-	2,4
D	*	+/-	2,4

*Not yet recognized in commonly isolated veterinary pathogens.
P = Plasmid. C = Chromosomal.

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