**Introduction**

The choice of effective empirical treatment of fever in the neutropenic host requires a sound knowledge of the local microbial epidemiology and the susceptibility patterns of pathogens. Since the list of possible pathogens can, at best, only be narrowed to a few most likely organisms, the use of empirical broad-spectrum antibiotic therapy in such situations is warranted. Indeed, the empirical use of broad-spectrum antimicrobial agents is further supported by the observation that the types of organisms that cause infections in the neutropenic host have changed over time. Amongst other factors, acquisition of resistance by microorganisms to the antimicrobial agents used for prophylaxis and therapy, has led to the need for continuous revision of empirical regimens.

A bacterial resistance to \( \beta \)-lactam antibiotics mediated by the production of drug inactivating enzymes has also influenced the selection of therapy. Various approaches towards empirical therapy in the neutropenic host have been adopted based on trends in bacterial resistance. One successful approach has been the development of potent agents such as the aminothiazolyl cephalosporins, monobactams and carbapenems that are relatively resistant to hydrolysis by the \( \beta \)-lactamases. These agents have been used as single agents or in combination with an aminoglycoside. Most combination antimicrobial regimens for empirical therapy include an aminoglycoside, but because of the untoward effects associated with these agents, coupled with the need for continued serum drug monitoring during therapy and the shift of infecting organisms towards Gram-positive bacteria, aminoglycosides have often been replaced by other agents.

An additional strategy to overcome drug resistance and expand the coverage against Gram-positive bacteria involves the use of \( \beta \)-lactamase inhibitors in combination with \( \beta \)-lactam antibiotics. The search for inhibitors of bacterial \( \beta \)-lactamases resulted in the discovery of clavulanic acid followed by other inhibitors such as sulbactam, olivanic acid, BRL 42715, penems, carbapenems and tazobactam. Structurally, tazobactam is a sulbactam derivative that has demonstrated highly potent activity against...
staphylococcal penicillinase, extended-spectrum β-lactamases and type II, III, IV and V β-lactamases of the Richmond & Sykes classification. The combination of tazobactam plus piperacillin, an extended-spectrum penicillin, has been shown to be active against several key β-lactamase-producing pathogens in febrile neutropenic patients.

Use of such combinations requires careful assessment of pharmacodynamics of the combination under conditions of changing drug concentrations, as occurs in the human host. This review will summarize the importance, in the neutropenic patient, of susceptibility of pathogens to β-lactam therapy and pharmacodynamic considerations for the use of β-lactam/β-lactamase inhibitor combinations such as piperacillin/tazobactam.

β-Lactamase-producing organisms in infection in the neutropenic patient

The pathogens responsible for infection in the neutropenic patient have changed significantly over the past decade, in part due to the use of antibacterial chemoprophylaxis as well as other interventions. Table I shows the bacteria frequently associated with infection in the neutropenic host. In the 1970s, Gram-negative pathogens, many of which produced β-lactamase (e.g., Escherichia coli), and Staphylococcus aureus, with associated high mortality rates, predominated. Bacteremic infections due to Pseudomonas aeruginosa had the highest mortality rates. In the late 1980s and through the 1990s there has been a change to a predominance of Gram-positive infections (particularly those caused by coagulase-negative staphylococci), largely as a result of the increasing use of indwelling central venous catheters. Indeed, Gram-positive cocci, particularly coagulase-negative staphylococci and viridans streptococci, have emerged as the main causes of infection in the neutropenic host. Athough infections caused by Gram-negative organisms are less prevalent in granulocytopenic patients, they continue to be associated with a high mortality rate.

In view of the importance of Gram-negative pathogens and their high mortality, broad-spectrum β-lactam antimicrobial agents have often been included in empirical antibiotic regimens in neutropenic patients. Unfortunately, the widespread and increasing incidence of bacterial resistance in many groups of pathogens has reduced their usefulness. β-Lactamases are a large family of enzymes with the ability to hydrolyse the β-lactam ring and are almost ubiquitous in bacteria. In bacteria, the genes for these enzymes may be found incorporated into the chromosome, on plasmids or on transposable elements.

Susceptibility to β-lactam antibiotics has been shown to be an important factor in the outcome of infection in neutropenic patients with Gram-negative bacteraemia. In a trial by the EORTC, cancer patients with bacteraemia were treated empirically with ticarcillin plus amikacin, cefotaxime plus amikacin, or azlocillin plus amikacin. While azlocillin plus amikacin was shown to be superior to

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<th>β-Lactamase-producing organisms in infection in the neutropenic patient</th>
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<th>A erobic G ram-negative bacilli</th>
<th>G ram-positive bacteria</th>
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<tr>
<td>E. coli</td>
<td>S. aureus (coagulase-positive)</td>
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<tr>
<td>K lebsiella spp.</td>
<td>coagulase-negative staphylococci</td>
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<tr>
<td>E nterobacter spp.</td>
<td>viridans streptococci</td>
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<tr>
<td>Serratia spp.</td>
<td>E nterococcus faecalis</td>
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<tr>
<td>P roteus spp.</td>
<td>Corynebacterium spp.</td>
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<td>P. aeruginosa</td>
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| Table I. Bacteria frequently associated with infection in the neutropenic host (adapted from Verhoef and Hughes et al.) |

<table>
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<th>Antibacterial agents</th>
<th>Resistant organisms</th>
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<tr>
<td>β-Lactams</td>
<td>Gram-negative bacilli with extended spectrum β-lactamases; P. aeruginosa</td>
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<tr>
<td>Carbapenems</td>
<td>P. aeruginosa; Stenotrophomonas maltophilia</td>
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<tr>
<td>A minoglycosides</td>
<td>Enterococci, Gram-negative bacilli</td>
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<tr>
<td>G lycopeptides</td>
<td>Enterococci; glycopeptide-resistant S. aureus</td>
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<tr>
<td>Fluoroquinolones</td>
<td>Staphylococci; Gram-negative bacilli</td>
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| Table II. Problems of emerging resistance to antibacterial agents of importance in the granulocytopenic patients (adapted from Shlaes et al.) |

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the other regimens, further analysis of the data showed that the response to treatment was significantly better (66% vs 21%) when the organism was susceptible to both the β-lactam and the aminoglycoside compared with when the organism was only susceptible to the aminoglycoside component but resistant to the β-lactam in the combination.

β-Lactamase inhibitors

Bacterial resistance due to β-lactamase production may be reversed by a β-lactamase inhibitor combined with a β-lactam. Three inhibitors currently in use are clavulanic acid, sulbactam and tazobactam.

The three main mechanisms by which β-lactamases may be inhibited are: competitive, non-competitive (time-dependent), and suicide inhibition. Livermore has described the kinetic steps of β-lactam inhibition (Figure 1). In the presence of β-lactamase, inhibitors can form an initial high affinity complex ([E–I]; Figure 1). However, this interaction fails to acylate the serine residue on the enzyme and does not result in opening of the β-lactam ring. This is a competitive inhibition process whereby the inhibitor competes with the enzyme for the substrate and inhibition might be overcome with excess enzyme concentration. Alternatively, a stable enzyme–inhibitor complex that fails to hydrolyse the inhibitor (non-competitive inhibition) can form. In this case, the inhibitor binds to a different site from the active site of the enzyme and, hence, remotely modulates the binding of the enzyme to substrate. The resultant enzyme–inhibitor complex is often very stable, but the process is reversible. Sometimes, the enzyme–inhibitor interaction yields a stable (E–I) complex that does not hydrolyse to release the active enzyme; then the inhibition is ‘suicidal’ in that enzyme can not be recycled and the active site is permanently perturbed (Figure 1). Thus, while competitive and non-competitive inhibition mechanisms are reversible processes, suicide inhibition is an irreversible process that results in the formation of a covalent non-hydrolysed bond between the enzyme and hydrolysed inhibitor (E–I* complex, Figure 1), stopping the hydrolytic potential of the enzyme.

The potency of the β-lactam/β-lactamase inhibitor combination depends on a number of factors. Measures of efficiency of inhibition include the IC₅₀ of the inhibitor (the inhibitor concentration required to achieve 50% loss of enzyme activity), affinity (measured by Kₐ), the induction potential of the inhibitor (to paradoxically induce the production of the enzymes) and the ‘turnover number’. The ‘turnover number’ refers to the number of molecules of inhibitor required to inactivate one molecule of β-lactamase enzyme. The more efficient suicide inhibitors have a low turnover number (i.e., need less inhibitor to inactivate a molecule of β-lactamase). Tazobactam has been reported to have a turnover number of 2–125 against the TEM-1 and TEM-2 β-lactamases, 50 against Enterobacter cloacae P99, and 4000 against Bacteroides fragilis TAL 3636, containing CcrA metallo-β-lactamases.

In-vitro activity of piperacillin/tazobactam vs pathogens in the immunocompromised host

Several reports have described the activity of tazobactam plus piperacillin against bacteria potentially encountered in infections in the immunocompromised host. Piperacillin plus tazobactam at fixed ratios of 8:1 or 4:1 is active against many strains of Gram-positive aerobic and anaerobic organisms such as Klebsiella pneumoniae, E. coli, Klebsiella oxytoca, Enterobacter aerogenes and Citrobacter diversus. Tazobactam shows no antagonism when tested in combination with various β-lactam antibiotics against species harbouring an inducible β-lactamase such as P. aeruginosa, Morganella morganii and E. cloacae. Fuchs & Barry reviewed the in-vitro activity of piperacillin/tazobactam against various Gram-positive and -negative organisms compared with that of imipenem or ceftazidime and observed a greater activity for piperacillin/tazobactam, especially against P. aeruginosa.

Integration of pharmacokinetics and pharmacodynamics concepts in the design of dosage schedules for piperacillin plus tazobactam

Studies in-vitro and in animal models of infection and in humans suggest that the time for which serum concentrations of β-lactams remain above the MIC is linked to a good outcome in the treatment of infections due to aerobic Gram-negative bacilli. For β-lactamase inhibitor/β-lactam combinations, concentrations of the β-lactam will exceed the MIC derived from in-vitro testing.
of the combination but concentrations of the inhibitor fall below the breakpoint concentrations used for in-vitro susceptibility (sulbactam, 8 mg/L; clavulanate, 2 mg/L; tazobactam, 4 mg/L) 2–3 h after a dose.

The above raises the question of whether there are prolonged effects of the inhibitor that allow for a longer duration of bactericidal activity of the β-lactam. The paradigm may be resolved through a hypothesis generated from data presented above. If the goal is to inactivate β-lactamases to reduce the MIC of the organism to accepted breakpoints for the β-lactam, one ought to deliver an amount of inhibitor at the initiation of therapy that should be adequate for several hours.

There is some in-vitro evidence of a ‘post-β-lactamase inhibition effect’. This phenomenon, similar to a post-antibiotic effect, is an effect of a β-lactamase inhibitor persisting beyond a period of exposure. Using an adaptation of classical methodology for evaluation of the post-antibiotic effect, the effect of transient exposure of β-lactamase-producing bacteria to tazobactam alone or with piperacillin on the bactericidal activity of piperacillin alone was recently explored in a β-lactamase-producing strain of E. coli. This strain was incubated with tazobactam or tazobactam plus piperacillin for 1 h, then centrifuged and the bacterial pellet resuspended in pre-warmed growth medium containing no drug or piperacillin alone. Pre-incubation with tazobactam alone and, in particular, in combination with piperacillin, resulted in piperacillin-induced killing of the strain during the second exposure period; in contrast, bacteria not exposed to tazobactam were not killed by piperacillin. These data suggest that, for β-lactamase-producing bacteria, exposure to tazobactam can lead to a prolonged period of susceptibility to piperacillin-induced bacterial killing, even when concentrations of the inhibitor are no longer present.

The amount of β-lactamase inhibitor required for potentiation of a β-lactam in vitro can be compared with the amount of exposure to the inhibitor in vivo, as expressed by an area under the serum concentration curve (AUC) or mean serum concentration. Figure 2 compares the concentrations of β-lactamase inhibitors tested at NCCLS susceptibility breakpoints with the mean concentration of the inhibitor in vivo for the regimens shown. Mean concentrations were derived using parameters previously reported. For sulbactam and tazobactam, mean concentrations in vivo exceeded the breakpoint concentrations used for sensitivity testing; in contrast, concentrations for clavulanate are approximately 65% of those used in in-vivo testing.

Studies in an in-vitro pharmacodynamic model

Our group has investigated the pharmacodynamics of β-lactam/β-lactamase combinations in an in-vitro model that allows for simulation of human pharmacokinetic properties of the drugs. The absence of host defences in the model is consistent with the clinical setting of a neutropenic patient.

The assumption that β-lactams and β-lactamase inhibitors must have matched pharmacokinetic properties for pharmacodynamic effects has been tested in vitro and in vivo. Our initial studies examined cefoperazone plus sulbactam for which the elimination half-lives differ by two- to four-fold. In an in-vitro hollow fibre model, it was shown that administration of cefoperazone 2 g or 4 g plus sulbactam 2 g, every 12 h, was effective against a strain of E. coli containing an extended-spectrum β-lactamase; this efficacy was observed despite prolonged periods of undetectable concentrations of sulbactam in the model. Others have shown the efficacy of combinations of β-lactams and β-lactamase inhibitors with disparate pharmacokinetic properties in animal models of infection.

Studies with isogenic strains of E. coli with differences in β-lactamase production allow the effect of dosage regimen of a β-lactamase inhibitor on pharmacodynamic properties to be studied. In studies with piperacillin/tazobactam, an isogenic pair of E. coli with one of the members having the TEM-3 extended spectrum β-lactamase, were tested. The β-lactamase-negative (wild-type) organism had an MIC of piperacillin of 4 and 2 mg/L when tested alone or in combination with tazobactam 4 mg/L, respectively. The TEM-3-containing isogenic strain was resistant to piperacillin (MIC = 128 mg/L), but was susceptible to piperacillin 2 mg/L in the presence of tazobactam 4 mg/L. Both strains were tested in the model against a fixed exposure of 12 g/day of piperacillin alone or in combination with 1.5 g tazobactam. The two regimens of piperacillin plus tazobactam tested were 3/0.375 g every 6 h or 4/0.5 g every 8 h (Figure 3). Piperacillin 3 g every 6 h...
resulted in a >99.9% kill of the piperacillin-susceptible strain by 6 h and throughout the duration of the experiment. Only a transient reduction followed by regrowth occurred in the TEM-3-containing strain. When the combination of piperacillin plus tazobactam was tested against the \(\beta\)-lactamase-negative strain, there was no enhancement of effect (data not shown), as might be expected. However, in experiments with the 3.0/0.375 g every 6 h and 4.0/0.5 g every 8 h regimens, the combination effectively restored the activity of piperacillin to that seen for the \(\beta\)-lactamase-negative strain. Piperacillin plus tazobactam, at either dosage regimen, was also active against piperacillin-resistant \(S.\) aureus in this model. These experiments suggest that extended dosage intervals are possible when large doses (as measured by the AUC) of an inhibitor is supplied over the course of the treatment.

Summary

Antibiotic resistance is a continuing problem in the treatment of hospital-acquired infections. Infections in neutropenic cancer patients require rapid initiation of regimens active against key pathogens. Combinations of \(\beta\)-lactam/\(\beta\)-lactamase inhibitors such as piperacillin plus tazobactam provide broad coverage against these pathogens. Studies in a pharmacodynamic model that is analogous to infection in a neutropenic patient show that current doses restore the activity of piperacillin against resistant organisms.

References


**β-Lactam/β-lactamase inhibitors and neutropenia**


