CHAPTER II

LITERATURE REVIEW

Hospital-acquired pneumonia

1. Definition and diagnostic strategies

In 2005, The American Thoracic Society (ATS) and the Infectious Disease Society (IDSA) published a consensus statement for defining, diagnosis and management of hospital-acquired, and ventilator-associated pneumonia.[23] Hospital-acquired pneumonia (HAP) is defined as pneumonia that occurs 48 hours or more after admission, which was not incubating at the time of admission. Some of the HAP patients have ventilator-associated pneumonia (VAP), which is diagnosed if the onset has occurred $\geq$ 48 h after the initiation of intubation. Although not included in this definition, some patients may require intubation after developing severe HAP and should be managed similar to patients with VAP.[23]

The diagnosis of HAP is difficult because there is no method for obtaining a diagnosis that is reliable in all cases, and most studies of patients have involved clinical diagnosis, with sputum culture, but bronchoscopy has been used less often. For suspecting HAP based on the presence of a new lung infiltrate or progressive radiographic infiltrate plus at least two of three clinical features (fever greater than $38^{\circ}C$, leukocytosis or leukopenia, and purulent secretions.)[26] A more definitive diagnosis can be obtained based on histopathologic examination of lung tissue, observation of rapid cavitation of lung infiltrate in radiographs, positive results from pleural fluid culture, or the isolation of pathogens from blood and sputum with no other identifiable source. However, both diagnostic approaches present potential problems for accurate diagnosis. The early clinical diagnostic may be too sensitive, whereas the more definitive diagnostic measures are costly, require skilled operators and specialized laboratories, and do not always produce accurate, definitive results.[23] These inherent problems mean that a substantial number (approximately 30%) of diagnoses are incorrect.[28] An additional clinical tool for diagnosing pulmonary infection is the Clinical Pulmonary Infection Score (CPIS). The CPIS combines clinical, radiographic, physiologic, and microbiologic data into a numerical score that correlates with the presence of pneumonia. A CPIS score greater than 6 has a good correlation with the presence of pneumonia diagnosed bronchoscopically or non-bronchoscopically.[29] However, other studies have found the CPIS to have a sensitivity of 77% and a specificity of 42%, making it less useful.[26] Subsequent work has suggested that a modified CPIS score of 6 or less is a good predictor of the ability to discontinue antibiotic therapy after 3 days for patients with a low suspicion for pneumonia and who are otherwise clinically improving.[26]

2. Etiology and epidemiology

HAP and VAP may be caused by a wide spectrum of bacterial pathogens, may be polymicrobial, and are rarely due to viral or fungal pathogen in immunocompetent hosts. Common pathogens include aerobic gram-negative bacilli, such as *P.aeruginosa*, *Escherichia coli, Klebsiella pneumoniae,* and *Acinetobacter* species. Infections due to gram-positive cocci, such as *Staphylococus aureus*, particularly methicillin resistant *S. aureus* (MRSA), have been rapidly emerging in the United States.[30] Pneumonia due to *S.aureus* is more common in patients with diabetes mellitus, head trauma, and those hospitalized in ICUs. The frequency of specific multidrug-resistant pathogens causing HAP may vary by hospital, patient population, exposure to antibiotics, type of ICU patient, and changes overtime, emphasizing the need for timely, local surveillance data.[31,32]

Epidemiologic estimates suggest that HAP occurs in 0.5% to 5% of all hospitalized patients. It is much more frequent among mechanically ventilated patients, occurring with the frequency of 15% to 25% in this important subgroup.[33] While the risk of dying among hospitalized pneumonia patients is much higher than the average risk of hospitalized patients, it has been difficult to determine the attributable mortality associated with HAP from mortality attributable to underlying conditions or the primary reason for hospitalization.[34] At least for VAP, it appears that crude mortality rates of 30% to 70% include approximately 15% to 50% attributable mortality specifically related to the VAP episode.[35] This risk of mortality may be different depending on the timing of VAP and the infecting organism, because pseudomonal and staphylococcal pneumonia have significantly higher mortality rates than do other forms of VAP. Increased mortality rates were also associated with bactereremia, especially with *P.aeruginosa* or *A.baumannii*, medical rather than surgical illness, and treatment with ineffective antibiotic therapy.[36,37] In USA, the development of VAP among mechanically ventilated patients adds approximately 7 to 9 additional hospital days and between $12,000 and $40,000 additional direct healthcare costs.[33]

In Thailand, 12% of hospitalized patients develop nosocomial infections, with nosocomial pneumonia infections accounting for 15% of these (1.8% of all hospitalized patients), In Thailand, and particularly in its large cities, nosocomial pneumonia is primarily caused by MDR gram-negative bacteria.[38]

3. Antibiotic treatment of hospital-acquired pneumonia

A management guideline was recently published by ATS/IDSA.[23] Antibiotic therapy for HAP or VAP depends on the likelihood of MDR pathogens. This can be clinically decided based on the duration of hospitalization before the development of pneumonia, with patients

who have been hospitalized at least 5 days having significantly greater risk for MDR pathogens.[31] For patients with early onset pneumonia (<5 days), antibiotic monotherapy may be adequate using an appropriate cephalosporin, quinolone, or extended-spectrum penicillin.[41,42] For patients with late-onset pneumonia, the likelihood of MDR pathogens is greater and therefore combination antibiotic therapy is preferred. This may include an anti-pseudomonal cephalosporin or carbapenem or anti-pseudomonal penicillin in combination with an anti-pseudomonal fluoroquinolone or aminoglycoside.[42,43] For patients with MRSA infections, either linezolid or vancomycin is considered appropriate. While linezolid has better respiratory tissue penetration and has been associated with improved outcomes in MRSA pneumonia, its superiority for improving clinical outcomes remains to be proven when compared to high-level vancomycin as should be used for MRSA pneumonia.[44] With effective therapy, improvement should be evident within 42 to 72 hours, most frequently in terms of oxygenation. For patients who do not respond within this time, consideration should be given to other organisms, whether there is an alternative diagnosis to explain the suspected infection, or whether another complicating factor is present (lung abscess or emphysema, drug fever, etc.), that has influenced the response to therapy. If patients receive an initially appropriate antibiotic regimen, efforts should be made to shorten the duration of therapy from the traditional 14 to 21 days to periods as 7 days, provided that patient has a good clinical response with resolution of clinical features of infection.[23]

In many countries, one of the more commonly used antibiotics in empiric therapy is a β-lactam with antipseudomonal activity, in particular ceftazidime. However, it has recently become clear that high levels of resistance in *Klebsiella* and *Enterobacter* spp. and *Escherichia coli* are a problem in some Asian countries, e.g., Thailand and Japan.[38] This has made treatment of pseudomonal pneumonia with ceftazidime very difficult. As a result, many countries have switched in the last couple of years to empiric therapy with a β-lactam/β-lactamase inhibitor combination, such as cefoperazone/sulbactam or piperacillin/tazobactam. In vitro studies have shown that the addition of a β-lactamase inhibitor to a β-lactam restores antimicrobial activity against pathogens that were resistant to the β-lactam alone.[45,46]

Cefoperazone is a third-generation cepholosporin that is comparatively stable in the presence of β-lactamase of some *Enterobacter* spp. and has a wide spectrum of activity, including gram-negative aerobes and anaerobes. It is also one of the few cephalosporins with activity against *P.aeruginosa*. When cefoperazone combined with the β-lactamase inhibitor sulbactam, the combination is active against β-lactamase producing gram-positive staphylococci in addition to many of the gram-negative organisms with plasmid-mediated β-

lactamase.[8] In Thailand; Suwangool P. et al.[47] performed a study in three hospitals to assess its activity in a clinical setting. They recruited 24 patients with nosocomial pneumonia. The most common causative agent was *P.aeruginosa* (37.5% of cases), followed by *K.pneumoniae* and *Acinetobacter* spp. (16.7% each). The patients were treated with cefoperazone/sulbactam 1-2 g twice daily for a mean duration of 13 days. The results of therapy were encouraging, with a clinical response being seen in 71% of patients (63% cure, 8% improvement). The microbiologic response showed eradication in 67%, persistence in 29% and superinfection in only one patient (4%).

In China, Li et al.[48] examined the efficacy of cefoperazone/sulbactam compared with the third-generation cepholosporin cefotaxime in a randomized, open-label study in the treatment of moderate to severe bacterial infection in hospitalized patients. Of the 207 patients enrolled in the study, 95 (46%) had a respiratory tract infection, of which 67 (70%) were pneumonia. Patients received either cefoperazone/sulbactam (2-4 g/day) or cefotaxime (6-12 g/day). The overall response rate for the β-lactam/β-lactamase inhibitor combination was 91.1% (67.6% cure, 23.5% improvement) compared with 84.8%(54.5% cure, 30.3% improvement) for cefotaxime.

The combination cefoperazone/sulbactam has also demonstrated efficacy in treating pneumonia in febrile cancer patients undergoing chemotherapy. A total of 514 patients enrolled in the study and received cefoperazone/sulbactam for a total of 673 episodes of fever. Of these, 83 (12.3%) were confirmed as resulting from nosocomial pneumonia. Cefoperazone/sulbactam was effective in treating these episodes, yielding a 61% success rate.[49]

4. Evaluation of response therapy

Resolution of HAP can be defined either clinically or microbiologically. Clinical end points such as improvement, resolution, delayed resolution; relapse, failure, and death can be defined. Using this approach, clinical improvement usually becomes apparent after the first 48-72 hours of therapy and, therefore, the selected antimicrobial regimen should not be changed during this time unless progressive worsening is noted.[23]

Appropriate respiratory tract culture can be used to define microbiologic resolution. Using serial cultures, end points can be defined, such as bacterial eradication, reinfections (infection with a new organism), recurrent infection (elimination, then return of original organism), or microbiologic persistence.[23,50]

Chest radiographs are of limited value for defining clinical improvement in severe pneumonia, and initial radiographic deterioration is common, especially among patients who

are bacteremia or who are infected with highly virulent organism. In addition, radiographic improvement often delays following clinical parameters, especially in the elderly and in individuals with coexisting disease.[51]

The changes of CPIS have been used in several studies to measure of resolution or deterioration among patients with VAP. Improvement in the CPIS occurring during the first 3 days of empiric treatment was associated with hospital survival whereas a lack of improvement in the CPIS predicted mortality. Inappropriate antibiotic treatment of VAP was also associated with a lack of clinical improvement in the CPIS, particularly in serial measurements of arterial oxygenation.[52]

Cefoperazone/sulbactam characteristics

1. Description[53]

The antibacterial component of cefoperazone/sulbactam is cefoperazone, a 3rd generation cephalosporin, which acts against sensitive organisms during the stage of active multiplication by inhibiting biosynthesis of cell wall mucopeptide. Sulbactam does not possess any useful antibacterial activity, except against *Neisseriaceae* and *Acinetobacter*. However, biochemical studies with cell-free bacterial systems have shown it to be an irreversible inhibitor of most important β-lactam produced by β-lactam antibiotic-resistant organism.

The potential for sulbactam's preventing the destruction of penicillins and cephalosporins by resistant organisms was confirmed in whole-organism studies using resistant strains in which sulbactam exhibited marked synergy with penicillins and cephalosporins. As sulbactam also binds with some penicillin-binding proteins, sensitive strains are also often rendered more susceptible to cefoperazone/sulbactam than to cefoperazone alone.

2. Antibacterial activity

The combination of cefoperazone and sulbactam is active against all organisms sensitive to cefoperazone. In addition, it demonstrates synergistic activity (up to 4-fold reduction in MIC for the combination versus those for each component) in a variety of organisms most markedly the following; *Haemophilus influenzae*, *Bacteroides* spp and *Staphylococcus* spp, *Acinetobacter calcoaceticus*, *Enterobacter aerogenes*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Morganella morganii*, *Citrobacter freundii*, *Enterobacter cloacae*, *Citrobacter diversus*.[54]

Cefoperazon-sulbactam is active *in vitro* against a wide variety of clinically significant organisms:

Gram-Positive Organisms: *Staphylococcus aureus*, penicillinase and nonpenicillinase-producing strains, *Staphylococcus epidermidis, Streptococcus pneumoniae* (formerly *Diplococcus pneumoniae*), *Streptococcus pyogenes* (Group A β-hemolytic streptococci), *Streptococcus agalactiae* (Group B β-hemolytic streptococci). Most other strains of β-hemolytic streptococci. Many strains of *Streptococus faecalis.*

Gram-Negative Organisms: Escherichia coli, Klebsiella sp, Enterobacter sp, Citrobacter sp, Haemophilus influenzae, Proteus mirabilis, Proteus vulgaris, Morganella morganii, Providencia rettgeri, Providencia sp, Serratia sp (including S. marcescens), Salmonella and Shigella spp, Pseudomonas aeruginosa and some other Pseudomonas sp, Acinetobacter calcoaceticus, Neisseria gonorrhoeae, Neisseria meningitidis, Bordetella pertussis, Yersinia enterocolitica.

Anaerobic Organisms: Gram-negative bacilli (including *Bacteriodes fragilis*, other *Bacteriodes* sp and *Fusobacterium* sp); gram-positive and gram-negative cocci (including *Peptococcus, Peptostreptococcus* and *Veillonella* spp); gram-positive bacilli (including *Clostridium, Eubacterium and Lactobacillus* spp)[55]

2. Susceptibility test

a) MIC determinations, serial dilutions of sulbactam/cefoperazone in a 1:1 or 1:2 sulbactam/cefoperazone ratio may be used with a broth or agar dilution method or E-test method.

b) Susceptibility disk zone size by Kirby-Bauer disk diffusion method: Use of a susceptibility test disc containing 75 mcg of cefoperazone and 30 mcg of sulbactam is recommended.

Table 1 Susceptibility Range of cefoperazone/sulbactam

MIC (μg/ml)		Susceptibility disk zone size (mm)	
Susceptible	$\leq$16	Susceptible	$\geq$21
Resistant	$\geq$64	Intermediate	16-20
		Resistant	$\leq$15

A report from the laboratory of "susceptible" indicates that the infecting organism is likely to respond to sulbactam/cefoperazone therapy, and a report of "resistant" indicates that the organism is not likely to respond. A report of "intermediate" suggests that the organism would be susceptible to sulbactam/cefoperazone if a higher dosage is used or if the infection is confined to tissues or fluids where high antibiotic levels are attained.

3. Pharmacokinetics

Cefoperazone: Cefoperazone is administered parenterally because it is not significantly absorbed from the GI tract. Peak serum levels of cefoperazone occur within 1-2 hours following an IM dose. The protein binding of cefoperazone is dependent on the serum concentration. The circulating drug is protein-bound at a concentration of approximately 93% at 25 mcg/ml, 90% at 250 mcg/ml, and 82% at 500 mcg/ml. It is distributed into most body tissues and fluids including the gallbladder; liver; kidney; bone; uterus; ovary; sputum; bile; prostatic tissue; adipose tissue; aqueous humor; and peritoneal, pleural, ascitic, and synovial fluids. It penetrates inflamed meninges and reaches therapeutic levels within the CSF. Cefoperazone crosses the placenta.[56]

Cefoperazone is metabolized hepatically and is excreted primarily in the bile. A small percentage is excreted in breast milk. In patients with normal renal and hepatic function, the elimination half-life of cefoperazone is 1.6-2.6 hours. In patients with biliary obstruction or hepatic cirrhosis, the elimination half-life increases as hepatic function declines and ranges from 3.4-7.1 hours. Urinary excretion is increased as hepatic function declines. Cefoperazone is removed by hemodialysis. Dosages should be adjusted accordingly. No significant differences have been observed in the pharmacokinetics of cefoperazone in renal failure patients.

Sulbactam: Sulbactam is administered parenterally. Peak serum levels of occur within 1 hour following an IM dose and immediately after IV infusion. Protein binding is approximately 38%. Drug is distributed into lungs; liver; gallbladder; prostate; middle ear effusions; bronchial secretions; maxillary sinus secretions; urine; and pleural, peritoneal, and synovial fluids. Approximately 15-25% of sulbactam is metabolized. The drug and its metabolites are excreted into the urine primarily via tubular secretion and glomerular filtration. In patients with normal renal function, the elimination half-life of sulbactam is 1-1.5 hours. The elimination half-life increases as renal function declines-up to 10-24 hours in patients with end-stage renal disease. Dosages need to be adjusted accordingly.[57]

Coadministration of sulbactam appears to have very little effect on the pharmacokinetics of ampicillin, penicillin G, or cefoperazone, suggesting that coadministration of sulbactam will not affect the usual dosing regimens for these β-lactam antibiotics.

Following IV administration over 15 minutes of a single 1, 2, 3, or 4 g of dose of cefoperazone, serum concentration of the drug average 138-158, 223-253, 331-340, and 506 μg/ml, respectively. The Cmax of drug following 1, 2 or 3 g of bolus dose average 140-200, 250-375 and 518 μg/ml, respectively.[56] The apparent volume of distribution of cefoperazone is approximately 10-13 L in adults. For sulbactam, following intravenous administration of 0.5 and 1 g sulbactam to healthy volunteers, peak serum plasma concentrations were approximately 20 and 40 μg/ml, respectively. The mean volume of distribution of sulbactam in central or plasma compartment is within 7.5-12 L in healthy volunteers.[57]

4. Therapeutic use

Cefoperazone-sulbactam is indicated for the treatment of the following infections when caused by susceptible organisms: Respiratory tract infections (upper and lower); urinary tract infections (upper and lower); peritonitis, cholecystitis, cholangitis and other intra-abdominal infections; septicemia; skin and soft tissue infections; bone and joint infections; pelvic inflammatory disease, endometritis, gonorrhea and other infections of the genital tract. Because of the broad spectrum of activity of cefoperazone-sulbactam, most infections can be treated adequately with this antibiotic alone. However, cefoperazone/sulbactam may be used concomitantly with other antibiotics if such combinations are indicated.[54]

5. Dosage and administration

Cefoperazone/sulbactam is commercial available in an injection form, as a fixed dose combination of cefoperazone and sulbactam in a 1:1 and 2:1 ratio. The 1:1 vial contains the equivalent of 500 mg and 500 mg of cefoperazone and sulbactam, respectively, while the 2:1 vial contains 1000 mg and 500 mg of cefoperazone and sulbactam, respectively. [54]

Adult dosage: The usual dosage is 2-4 g/day (1-2 g cefoperazone activity) given in equally divided doses every 12 hours. In severe or refractory infections the daily dosage of cefoperazone/sulbactam may be increased up to 8 g of the 1:1 ratio (4 g cefoperazone activity) or 12 g of the 1:2 ratio (8g of cefoperazone activity). Doses should be administered every 12 hours in equally divided doses. The recommended maximum daily dose of sulbactam is 4 g.[58]

In adult with hepatic disease dosage of cefoprerazone should not exceed 2 g without close monitoring of serum concentrations. Patients with renal dysfunction, the dosage regimen should be adjusted in patients with marked decrease in renal function (creatinine clearance of < 30 mL/min) to compensate for the reduced renal clerance of sulbactam. Patients with creatinine clearance 15-30 mL/min should receive a maximum of 1 g of sulbactam administered every 12 hours. (Maximum daily dosage of 2 g sulbactam), while patients with creatinine < 15 mL/min should receive a maximum of 500 mg of sulbactam every 12 hours.(maximum daily dosage of 1 g sulbactam).[58]

The importance of appropriate antibiotic dosing : Pharmacokinetic and pharmacodynamic considerations

The emergence of antibiotic resistance in common respiratory pathogens have led to a reevaluation of the selection of antibiotic dosing regimens. The primary goal of antimicrobial therapy is to provide adequate drug concentrations at the site of infection that will achieve bacterial eradication and clinical cure.

The convention of antibiotic dosing on a milligram-per-kilogram basis can be a poor measure of drug exposure, with wide variations in actual exposure among patients. The drug exposure can fluctuate greatly in patients receiving the same antibiotic regimen. As a result, dosing regimens based solely on milligram-per-kilogram values may be an inadequate method of determining the target drug concentrations that best correlate with bacterial eradication and clinical cure.

The better approach to determining appropriate dosing regimens and optimal drug exposure come from integrating the pharmacokinetic (PK) and pharmacodynamic (PD) parameters of a drug to determine its antimicrobial efficacy and resistance.[59,60]

1. Pharmacokinetics principle

Pharmacokinetics (PK) has been defined as follows by Holford and Sheiner:[61] "Pharmacokinetics encompasses the study of movement of drugs into, through and out of the body. It describes the processes and rates of drug movement from the site of absorption into the blood, distribution into the tissues and elimination by metabolism or excretion."

Application of PK principles allows describing the relationship between dose and drug concentration. This is one important component of the relationship between dosage regimen and time course of effect. The existence of the second component, the concentration-effect relationship (pharmacodynamic), is a fundamental hypothesis of pharmacology and has been

documented for many drugs. Therefore, by predicting the time course of drug concentration we have made one importance step towards predicting the time course of drug effect. If the same dosage regimen of a drug is given to different patients, there is true between subject variability in the observed drug concentration. A reason for these may be that patients differ in their ability to absorb or eliminate a drug. By including the variability of the PK parameters into a PK model, one can predict drug effect more precisely and optimize dosage regimen for the population.[62]

1.1 Pharmacokinetic Parameters

There are mathematical models that explain the pharmacokinetic principles that govern the input and elimination processes determining the final drug concentration. These parameters are clinically useful in designing safe and effective dosage regimens.[63]

1.1.1 Bioavailability (F)

Absorption is dependent on bioavailability (F) of the drug. Bioavailabiltiy is the percentage or fraction of the administered dose that reaches the systemic circulation of the patient. Examples of factors that can alter bioavailability include the inherent dissolution and absorption characteristics of the administered chemical form (e.g., salt, ester), the dosage form (e.g., tablet, capsule), the route of administration, the stability of the active ingredient in the gastrointestinal and the extent of drug metabolism before reaching the systemic circulation.[64]

1.1.2 Volume of Distribution (Vd)

Distribution refers to the exchange of drugs among the various body compartments. The distribution of the drug beyond the intravascular space depends on its molecular size, ionization at physiologic pH, water/lipid solubility, and the degree of binding to plasma proteins.[65]

The volume of distribution (Vd) for a drug or the apparent volume of distribution is the term used to relate the concentration of drugs in the body to the amount of drugs in the plasma. It is a theoretic measure that indicates the extent more than the site of distribution. The plasma volume of the average adult is approximately 3 L. Therefore, apparent volumes of distribution that are larger than the plasma compartment (>3L) only indicate that the drug is also present in tissues or fluids outside the plasma compartment.[66]

1.1.3 Plasma concentration (C)

Most clinical laboratory reports of drug concentrations in plasma (C) represent drug that is bound to plasma protein plus drug that is unbound or free. It is the free or unbound drug that is in equilibrium with the receptor site and is, therefore, the

pharmacologically active moiety. Thus, in the case of a drug with significant plasma binding, the reported plasma drug concentration indirectly reflects the concentration of free or active drug. The fraction of drug that is unbound (fu) does not vary with the drug concentration for most drugs that are bound primarily to albumin.[67]

Although many clinicians believe that monitoring free or unbound plasma concentration is clinical practice. The reasons are several and include the fact that assay procedures for free or unbound drug are not commercially available for many compounds. Furthermore, the assay procedures available for free drug concentrations are more expensive. Whereas in theory monitoring unbound drug concentrations should be clinically superior, there is little evidence demonstrating that monitoring unbound drug levels improves the correlation between the plasma concentration and the pharmacologic effect or therapeutic outcome.

1.1.4   Half-life (T1/2) and Elimination Rate Constant (Ke)

A drug half-life (T1/2) is the time it takes for the plasma concentration to fall by one half; the elimination rate constant (Ke) is the slope of the line formed when the natural logarithm of drug concentration versus time is plotted. These two parameters are important to remember when estimating the time to total drug elimination. Most drugs will be eliminated in approximately five half-lives. The steady state is the condition reached when the same amount of drug that enters a given compartment per unit of time is eliminated at the same rate from that compartment. Most therapeutic drug concentration refers to this state.[68]

1.1.5   Clearance (CL)

Clearance (CL) refers to the time it takes for a drug to be eliminated from the blood. It is the sum of all routes of elimination and is affected by changes in the function of the organs involved in the elimination or distribution of a drug. Clearance is expressed as a volume per unit of time. It is important to emphasize that clearance is not an indicator of how much drug is being removed; it only represents the theoretical volume of blood or plasma which is completely cleared of drug in a given period. The amount of drug removed depends on the plasma concentration of drug and the clearance.[68]

1.1.6   Creatinine clearance (Clcr)

Because many drugs are practically or totally eliminated by the kidney, an accurate estimation of renal function is an important component in the application of pharmacokinetics to designing drug therapy regimens. Creatinine clearance (Clcr) as determined by a urine collection and corresponding plasma sample is considered by many clinicians to be the most accurate test of renal function. The most common method used to determine Clcr from serum creatinine is the equation from Cockcroft and Gault. [69]

Clcr for males (ml/min) = (140-Age) (Weight)/(72) (Scr)   equation 1

Clcr for females (ml/min) = (140-Age) (Weight)/(72) (Scr) x 0.85   equation 2

Where age is in years, weight is in kg, and serum creatinine is mg/dL.

The two most critical factors to consider when using the above equations are the assumptions that 1) the serum creatinine is steady state and 2) the weight, age, and gender of the individual reflect normal muscle mass. For an obese patient, the ideal body weight (IBW) should be used in equation. This estimate can be based on ideal body weight equations.

Ideal Body weight for males (kg) = 50 +(2.3)(Height in inches > 60)   equation 3

Ideal Body weight for females (kg) = 45 +(2.3)(Height in inches > 60)   equation 4

As a clinical guideline approach is to make an adjustment for ideal body weight if the patient's actual weight is >120% of their ideal body weight.

1.2   Microbiology and Pharmacokinetics

Parameters for pharmacokinetic properties of antimicrobial agents are the same as for those of other therapeutic drugs. There is no particular issue in pharmacokinetic considerations with antimicrobial agents except that the target site is located in the infection site where microorganisms are living. The infection sites are distributed throughout the body, and an effective concentration of an antimicrobial agent is required to eradicate microorganisms in those infection sites where they are living.[73] The pharmacokinetic parameters related to microbiological activity of an antibiotic are

Peak drug concentration (Cp)

Half life (T1/2)

Area under the drug concentration curve (AUC)

Time period during which drug concentrations exceed MIC or MBC

1.3   Determination of drug concentration.

1.3.1   Drug sampling

In order to obtain useful and reliable information about drug concentrations, the sampling must consider the following factors:[71]

1. Route and method of administration: Intravenous antibiotics are usually administered intravenously over 15-60 minutes. Prolonged infusions and sampling

peaks for those compounds are obtained. In reversed phase HPLC the mobile phase is often mixture containing water, buffers, methanol, acetonitrile or tetrahydrofuran.

The stationary phase consists of small particles, which produce a bed with very high flow impedance. Consequently, very high pressures are necessary to force the mobile phase through the column. In reversed phase HPLC the stationary phase consists of silica gel with hydrocarbon chains that are bound to the surface. The polarity of the stationary phase depends on the length of the hydrocarbon chains. Common stationary phases contain C8 or C18 chains

A HPLC system consists of one or more pumps, an injection system, a column, a detector, and a computer. The pump pushes the mobile phase through the system with a constant or changing flow rate. The injector transports the sample into the mobile phase. The column is a stainless steel tube that contains the stationary phase.

The detector detects the compounds as they elute from the column by measuring response changes between the mobile phase alone and the mobile phase containing the sample. The electrical response from the detector is recorded and sent to a data system. A peak on the chromatogram is observed.

The most appropriate method of detection depends on the properties of the drug to be quantified. Besides mass spectrometers, ultraviolet (UV) detectors and fluorescence detectors are two of the most common detectors. A UV detector measures the ability of a sample to absorb light at one or several wavelengths. As many compounds contain conjugated electron systems that can act as chromophores and absorb UV light, this detector is widely applied. The solvents that make up the mobile phase should not absorb UV light at the same wavelengths as the analyte. The Beer-Lambert law describes the relationship between the intensity of the transmitted light and the concentration of the analyte in the solution.

For quantification of the analyte in the sample, the peaks in the chromatogram are evaluated by different methods. Two common methods are determination of the peak height or the peak area of the analytes. An internal standard is used to account for variations e.g. during sample preparation or due to inaccuracy in injection volume. The internal standard is added to each sample in a known concentration. The peak height or peak area of the analyte is then compared to the respective value of the internal standard. By this method the concentration ratio between the analyte and the internal standard, and finally the concentration of the analyte in the sample may be calculated.

1.3.3 Pharmacokinetic calculation by non-compartment analysis [73]

ports distant from the patient may decrease peak antibiotic concentrations. Samples should not be collected from the same site the drug is infused.

2. Timing: Sample collection should be performed after distribution of the drug into the vascular system is completed (e.g., one hour for aminoglycosides). The timing of the test sample must be as close to steady state as possible. In most clinical settings, this is performed after three to five half-lives have passed from starting antibiotic therapy. Peak and trough concentrations preferably should be obtained after the same dose, not before and after the same dose as is often done, until desired level is reached and no more changes in dose or interval are expected. Once the steady state has been reached, obtaining peak and trough levels with the same dose is reliable since all peak and troughs will be the same.

1.3.2   Analysis of plasma sample by High Performance Liquid Chromatography (HPLC) with UV detection. [72]

High performance liquid chromatography (HPLC) is a frequently used method for bioanalytical determination of drug concentrations. The drug to be analyzed is chromatographically separated from the other components of the sample and then quantified. Chromatography is a physical method of separation, the components partition between a mobile and a stationary phase. In HPLC the sample to analyze is dissolved in a solvent and then transported with the mobile phase under high pressure over the stationary phase. The solutes are separated due to differences in their affinity to the two phases. In normal phase HPLC, the stationary phase is more polar than the mobile phase, however reversed phase HPLC is more common in bioanalysis of antibiotics. In reversed phase HPLC the mobile phase is more polar than the stationary phase, and polar substances are elute faster than non-polar substances, as polar substances have less affinity to the stationary phase. Thus, reverse phase HPLC is most useful for non-polar drugs or compounds with low polarity at the chosen pH.

The mobile phase is usually a mixture of different solvents. In isocratic elution the mixture is the same during the whole analysis. If the use of a single mobile phase composition does not result in adequate separation of all compounds, often-gradient elution is advantage. In gradient elution the composition of the mobile phase changes in a pre-defined way during the analysis of each sample to improve the resolution or shorten retention times. This change can be continuous or stepwise. In gradient elution the strength of the mobile phase to elute the analyte is increased during the analysis. In reversed phase HPLC this means that the mobile phase becomes less polar. Therefore the retention times of compounds with very high affinity to the stationary phase are shortened and sharper

Non-compartment analysis (NCA) is a standard technique of PK analysis. NCA is easier to apply and relies on fewer assumptions than compartmental modeling. Standard NCA assumes linear PK, i.e. all transport processes are assumed to follow first-order kinetics. NCA does not provide a mechanistic description e.g. of saturable elimination. The PK parameters are calculated from the individual plasma concentration.

In NCA, for determination of the terminal half-life (T1/2), the slope (λz) of the terminal part of the time versus logarithmic plasma concentration curve was determined by linear regression. To determine the terminal slope, at least three of four observations should lie in this part of the curve. T1/2 was then calculated as ln2 divided by λz.

Figure 1.   Determination of terminal half-life by NCA
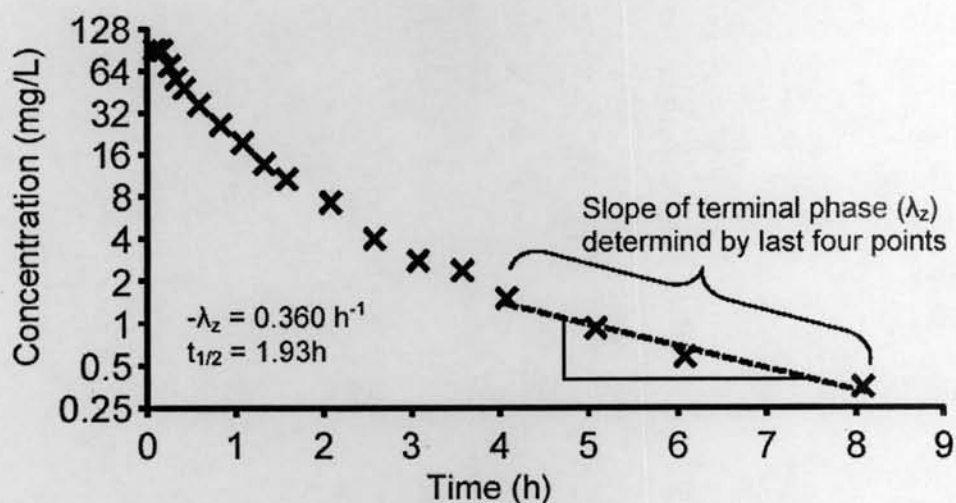
The area under the plasma (or serum) concentration time curve (AUC) was calculated by the trapezoid method. Total body clearance (CL_T) was determined by dividing the administered dose by the AUC in plasma. The area under the product of the concentration and time versus time curve is called the area under the first moment concentration time curve (AUMC). AUC is the area under the zero moment curves. Mean residence time (MRT) is the time that a drug molecule stays in the body, excluding the gastrointestinal tract. MRT is calculated as the ratio of AUMC divided by AUC. After intravenous dosing, volume distribution at steady-state (Vss) was calculated as MRT . CL_T. In this study, we used WinNonlin^{TM} (Pharsight, USA) for NCA.

2. Pharmacodynamics

Pharmacodynamic is the term used to reflect the relationship between measurements of drug exposure in serum, tissue, and body fluids and the pharmacological and toxicological effects of drugs. With antibiotic pharmacodynamic is focused on the relationship between concentrations and the antimicrobial activity against a pathogen. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) have been the major parameters used to measure the in vitro activity of antimicrobial against various pathogens.[11]

2.1 Patterns of antimicrobial activity

In general, antibiotic are classified into three classes on the basis of their antimicrobial killing patterns. These include concentration and time dependent killing.[10,74]

2.1.1 Concentration-dependent killing.

Concentration-dependent antibacterials display an increased rate and extent of bacterial kill with increasing drug concentration. This pattern is observed with aminoglycosides, fluoroquinolones, daptomycin, ketolides, metronidazone, and amphotericin B. The goal of a dosing regimen for these drugs would be to maximize concentrations. The peak level and the AUC should be the pharmacokinetic parameters that would determine efficacy.

2.1.2 Time dependent killing and minimal to moderate persistent effect

The antibiotics demonstrate high drug levels would not kill organisms better than lower concentrations. Futhermore, organism regrowth would start very soon after serum levels fell below the MIC. This pattern is observed with β-lactams, macrolides, clindamycin, and flucytosine. Therefore, the duration of time that serum levels exceed the MIC should be the major pharmacokinetic parameter determining the efficacy.

2.1.3 Time dependent killing and prolonged persistent effects

These drugs are also characterized by time-dependent killing, but can prevent any regrowth during the dosing interval. This pattern is observed with azithromycin, tetracyclines, quinupristin-dalfopristin, glycopeptides, and fluconazole. The AUC should be the primary pharmacokinetic parameter that would determine in vivo efficacy

2.2 Pharmacokinetic/pharmacodynamic parameters

Pharmacokinetics/pharmacodynamics (PK/PD) is the combination of the dose-concentration relationship with the concentration-effect relationship. PK and PD are

combined in order to describe the time course of drug effect for a chosen dosage regimen. Knowledge about the time course of drug effect allows one to compare and optimize dosage regimens. This is one important clinical application of PK/PD model.

By using the MIC as a measure of the potency of drug-organism interactions, the pharmacokinetic parameters determining efficacy can be converted to PK/PD parameters. It can be simplified to the peak concentration (peak)/MIC ratio, the length of time the serum concentration remains above the MIC (T>MIC) and the area under the curve (AUC)/MIC.[75,76]

The largest number of studies in animal addressing the time above MIC has consistently been the only PK/PD parameter correlating with the therapeutic efficacy of the various β-lactam antibiotics. Studies demonstrate that antibiotic concentrations do not need to exceed the MIC for 100% of dosing interval to obtain a significant antimicrobial activity. Time above MIC is also the parameter correlating with efficacy of macrolides, clindamycin and flucytosine.[13,14]

The AUC/MIC and peak/MIC ratios have been the PK/PD parameters that correlate with efficacy for aminoglycosides and fluoroquinolones. Most studies have shown slightly better correlation with the AUC/MIC ratio than with the peak/MIC ratio. Peak/MIC ratios appear to be more important in infections where the emergence of resistant subpopulation is a significant risk and for drugs that act on the cell membrane, such as daptomycin and amphotericin B.[77]

2.3 Pharmacokinetic/pharmacodynamic variation in pathophysiological states

To achieve the desired pharmacodynamic in target in antimicrobial selection, clinican must consider more than only an organism's susceptibility as reflected in the MIC. To develop individualized therapy, one must also consider patient-specific pharmacokinetic variation. Significant inter-patient variability may exist in drug absorption, distribution, metabolism, and elimination that affect the ability to achieve pharmacodynamic targets at conventional doses. Alterations in absorption, protein binding, tissue perfusion, and other factors which have impact on active drug concentrations ,and potential clinical efficacy. Commonly, encountered situations, in which pharmacokinetics may be vastly altered and dosing individualization, may be necessary including renal and hepatic dysfunction, critical illness, sepsis, burns, and obesity.[78]

2.3.1 Renal Dysfunction

The most common reason that antibiotic doses must be adjusted is for a reduction in drug elimination secondary to organ dysfunction. Age-related decline in renal function accounts for an approximately 5-10% reduction in glomerular filtration per

decade beyond the age of 30. Other causes of reduction in renal clearance include acute or chronic renal dysfunction frequently seen in diseases such as diabetes or heart failure. When doses are not adjusted, accumulation results in supratherapeutic concentrations, which increases the likelihood of concentration-dependent adverse effects.[78]

Antibiotics that require the dose adjustment in reduced renal clearance vary by class. Aminoglycosides and vancomycin are both eliminated primarily by glomerular filtration, and extensive literature is dedicated to dosing recommendations for these agents in various degrees of renal function. For aminoglycosides, nephotoxicity has been associated with drug accumulation, so it is essential to provide appropriated adjustment to minimize the likelihood of this effect. Many β-lactam antibiotics are eliminated renally, and doses must be adjusted for reduced glomerular filtration. Simple equations such as those described by Cockroft and Gault or Jelliffe may be used to estimate a patient's creatinine clearance. A reduction in dose while maintaining an interval that ensures adequate T>MIC may be more desirable.

2.3.2 Hepatic impairment

In patients with hepatic disease, clearance of many drugs may be impaired; however, the magnitude of the impairment in metabolic function has not been quantified by any single parameter. Adjustment in dosing schedule should be considered for drugs that are substantially cleared by the hepatobiliary system, such as clindamycin and antituberculous agents. Metabolic clearance may be further reduced in end-stage hepatic cirrhosis. The greatest risk of excessive accumulation exists in patients with both hepatic and renal impairment. When renal clearance is reduced in the presence of concomitant liver dysfunction and ascites, elimination half-life of many antibiotics is prolonged, and potentially increasing the risk of adverse effects. In this situation, pharmacokinetic patterns are highly variable, and specific dosing recommendations are difficult to predict.[79]

2.3.3 Clinical illness/sepsis

Critically ill patients may have pathophysiologic conditions that alter drug absorption, distribution, and clearance. In general, altered distribution and elimination have been observed with a number of antibiotic classes, requiring special consideration of drug selection and dosing in these patients.[80]

A decrease in plasma albumin is also seen in critical illness, potentially leading to an increase in the free fraction of drug that is ordinarily highly bound to this protein. An increase in the volume of distribution for drugs may occur, resulting from a combination of fluid resuscitation, renal failure, and cardiac compromise and vascular congestion. For antimicrobial agents extensively metabolized by the liver, metabolism will be

affected both by changes in liver perfusion and protein binding. Renal failure has been reported with up to a 23% incidence in ICU patients.[81] Many antimicrobial agents depend on renal elimination, and the elimination half-life for these drugs may be prolonged. Because such variability has been demonstrated in critically ill patients, target drug monitoring is often appropriate, when available, to assure the desired pharmacodynamic targets are achieved.

The pathophysiologic changes in critically ill patients results in unpredictable pharmacokinetics of β-lactam antibiotics. Impaired renal perfusion and hepatic metabolism can lead to drug accumulation and higher risk of toxicity. Conversely, fluid resuscitation and retention can increase volume of distribution and result in lower serum concentrations of antibiotics. In an animal model of trauma, aztreonem clearance was initially decreased, followed by a sharp increased of almost 50% of baseline, while volume of distribution was decreased throughout the first week after injury. Volume of distribution of both aztreonam and imipenem-cilastatin were significantly increased in a study of trauma patients with pneumonia; however, clearance was significantly prolonged only for aztreonam. This variation in pharmacokinetics suggests that perhaps adjustments of both dose and interval of β-lactam antibiotics need to be individually considered in critically ill patients.

Intermittent boluses of β-lactam antibiotics in ICU patients can result in variable plasma concentrations, with unpredictable T>MIC. Continuous infusion of ceftazidime has been shown to produce more consistent concentrations in critically ill patients and was found to be more efficacious in killing *P.aeruginosa* in an in vitro pharmacokinetic model. However, at many situations, continuous infusion is not a routine method of antibiotic administration. In the ICU, resistance of infecting organisms may be observed more frequently. Therefore, optimal T>MIC may not be achieved with traditional dosing, and combination therapy may be needed to achieve optimal pharmacodynamic target.[82]

2.4 Pharmacodynamic simulations

PD simulations are used to determine the probability of successful microbiological or clinical outcome for a specific antimicrobial dosage regimen. The microbiological outcome of antibiotic treatment is related to the ability of an antibiotic to kill the pathogens causing the infection or to inhibit their growth. Besides the right choice of antibiotic, a sufficient exposure to the antimicrobial is necessary for successful microbiological outcome. As protein biding was shown to have an impact on microbiological activity of antibiotics. Only free (non-protein bound) drug is considered microbiologically active.[83] PD simulations has many applications, including use for establishing optimal dosing regimens for old drugs, for

developing new antimicrobials and formulations, for setting susceptibility breakpoints and for providing guidelines for empirical therapy.[84]

2.5 Monte Carlo simulation in the field of PKPD

Monte Carlo simulation (MCS) was originally called "statistical sampling". MCS is a stochastic simulation method that uses random numbers to simulate data. Therefore, the exact result of a single experiment cannot be predicted. This differentiates MCS from deterministic simulations. In deterministic simulations, one specific input will always give the same result and therefore the simulations are fully predictable. The name Monte Carlo simulation refers to the famous casino and was chosen because of the randomness and repetitions involved in the simulations. MCS has been used for very different purposes such as the study of the properties of the neutron in 1930, and in the development of the hydrogen bomb in the 1950s. The first use of MCS for selection of antibiotic doses and setting susceptibility breakpoints was presented in 1998 by Drusono et al.[85] They showed that MCS is a valuable tool in rational dose selection for phase II/III clinical trial.[86]

The results of MCS analyses estimate what is probable, rather than defining what is possible. MCS are sampling experiments for estimating the distribution of an outcome that is dependent on multiple probabilistic input variables. For example, MIC values obtained from institution or region and the 24 h AUC value from patients are considered as input variables. Random values across input variable that conform to their probabilities are generated, and then an output is calculated (e.g., AUC/MIC ratio). Each individual output, that is calculated, is then plotted in a probability chart. MCS methodology demonstrates the range of possible outcome and probability associated with each.[87]

Although MCS cannot predict the concentration time profile for a new subject, it can predict the expected range of concentration time profiles for a population of individuals for chosen dosage regimens. Based on this expected range profiles, the probability of attaining (PTA) a PKPD target for dosage regimen of interest can be predicted. This PTA depends on the antibiotic concentrations in the patient population and on the susceptibility of the infecting pathogen. There is variability in the concentration time profiles between patients and in the bacterial susceptibility to an antibiotic (described by the MIC)

To describe the distribution of expected antibiotic concentrations for a chosen dosage regimen, the concentration time profiles for a large number of virtual subjects (e.g. 10,000) are simulated. Simulation 10,000 subjects provide a robust prediction of PTA. Those simulations are based on a population PK model that must have adequate predictive performance to yield unbiased estimates for the PTA.

The PKPD targets for antibiotics are based on the MIC of the pathogens. Therefore, the PTA is predicted for a range of MICs. The PKPD indices (e.g. fT>MIC for beta-lactam of fAUC/MIC for quinolones) are then calculated for the 10,000 subjects at each MIC within this range. The values of the PKPD indices are compared to the PKPD target (e.g. fT>MIC at least 50% of dosing interval) for all 10,000 simulated subjects who attained the target at each MIC. The PTA at each MIC is then derived by calculating the fraction of subjects who attained the target at each MIC.

The highest MIC for which the target is attained by at least 90% of the simulated subjects is often defined as the PKPD breakpoint.[88] Therefore the PKPD breakpoint is the highest MIC, for which the probability of successful treatment with the chosen dosage regimen is ≥ 90%. If a patient is infected by pathogens with an MIC higher than the PKPD breakpoint, another dosage regimen should be chosen. Importantly, the PKPD breakpoint is determined in a different way compared to the susceptibility breakpoints, which are set by the CLSI (Clinical and Laboratory Standard Institute). Consequently, the PKPD breakpoint and the susceptibility breakpoints set by those organizations may differ by more than a factor of 4.

Figure 2   PTA vs. MIC profile and derivation of the PKPD breakpoint
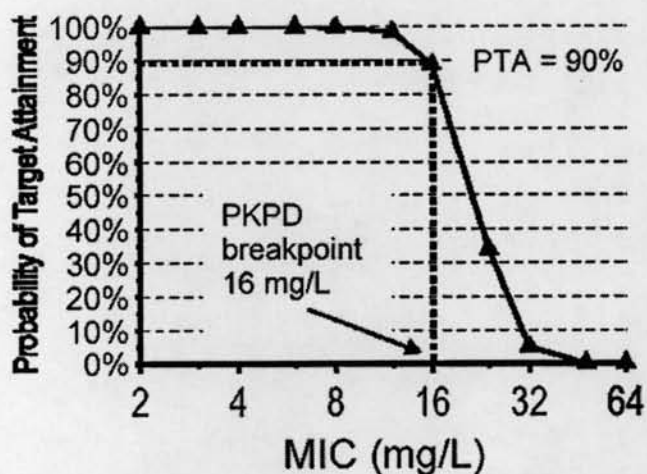
To put the PTAs into clinical perspective, the PTA expectation value (also called cumulative fraction of response) is calculated. The PTA expectation value is an estimate of the proportion of the population that will achieve the PKPD target, for a specific drug dose and a specific population of microorganisms. MIC distributions of pathogen of clinical interest can be obtained from published studies where large numbers of isolates were collected and their MICs determined. Alternatively, the MIC distribution of a local hospital can

be used. The PTA expectation value is calculated by multiplying the PTA at each MIC by the fraction of the population of microorganisms at each MIC. Ideally, a PK model, which has been determined in the patient population of interest, is combined with the MIC distribution typically observed in those patients at a local hospital. The PTA expectation value can then be used to predict the probability of microbiological of clinical success in this local hospital.

Figure 3   Calculation of the PTA expectation value based on the PTA vs. MIC profile and the expected MIC distribution
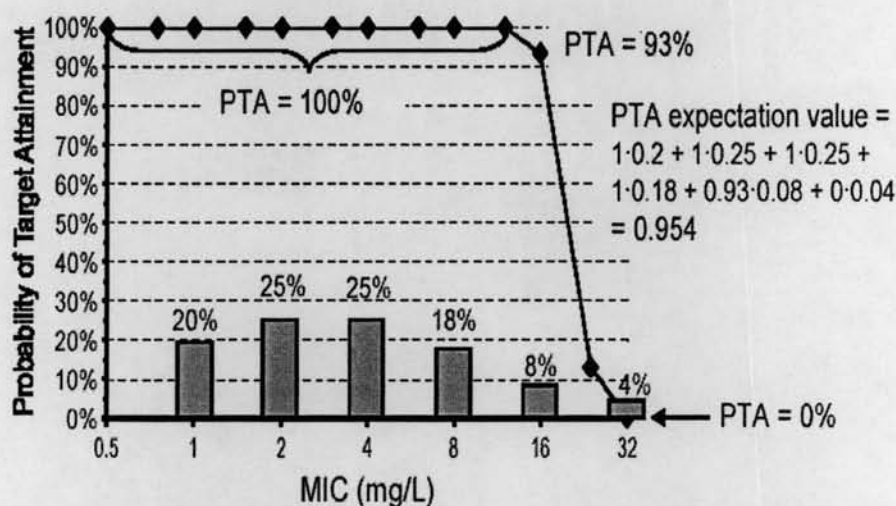
3. The Pharmacodynamics of β-lactam antibiotics

3.1   Pharmacodynamic concept

For β-lactam, in vitro and animal studies have demonstrated that the amount of time in which the free or non-protein bound drug concentration exceeds the MIC of the organism (fT>MIC) is the best predictor of bacterial killing and microbiologic response. The studies have consistently shown that free β-lactam concentrations do not have to remain above the MIC for the entire dosing interval. They have also demonstrated that the fraction of the dosing interval for maximal bacterial effect varies for the different types of β-lactams. Although the precise fT>MIC varies for different drug-bacteria combination, bacteriostatic effects are typically observed when the free drug concentration exceeds the MIC for 35-40%, 30% and 20% of the dosing interval for the cephaolsporins, penicillins and carbapenems,

respectively. Near-maximum bactericidal effects require 60-70%, 50% and 40% fT>MIC, respectively, for these β-lactam classes.[13,14,15]

For β-lactam antibiotics administered intravenously or with rapid oral absorption, estimates of the percentage of time above MIC (%T>MIC) can be determined by entering pharmacokinetic parameters and MIC values into the following equation:[89]

$$\% \ T>MIC = \ln \frac{Dose}{Vd.MIC} \ \times \ \frac{T1/2}{0.693} \ \times \ \frac{100}{DI} \qquad \text{equation 5}$$

Where ln is the natural logarithm, Dose is the intermittent dose in milligrams, Vd is the apparent volume of distribution in liters, MIC is the minimum inhibitory concentration in micrograms per milliliter, T1/2 is the half-life in hours, and DI is dosing interval in hours.

In case of pharmacodynamic exposures, as measure by % T>MIC for free drug, can be determined by the equation: [89]

$$\% \ free \ T>MIC = \ln \frac{(Dose \ . \ f)}{Vd.MIC} \ \times \ \frac{T1/2}{0.693} \ \times \ \frac{100}{DI} \qquad \text{equation 6}$$

Where f is the fraction of unbound drug.

3.2 Factor affecting

3.2.1 Postantibiotic effect

The postantibiotic effect (PAE) is the suppression of bacterial growth that persists when drug is removed after a short exposure of microorganism to an antimicrobial. All antimicrobials appear to have a PAE to gram-positive bacteria in vitro.[90] The mechanism of PAE is not well understood. It may relate to non-lethal damage of the bacteria or limited persistence of the drug at a cellular site of action. Factors that have been shown to influence the PAE include the type of organism, the dose and concentration of antibiotic, the duration of exposure, and the size of the bacterial inoculum.

In vitro, by the viable count, the PAE of penicillins and cephalosporins to gram-positive bacteria is consistently 1-3 hours. Imipenem and meropenem are the only β-lactams that demonstrates a PAE to gram-negative bacteria, primarily P.aeruginosa. The PAE of these carbapenem to P.aeruginosa appears to be strain-dependent and varies up to 2 hours in length depending on the particular strain.[90]

In a neutropenic mouse thigh model, all β-lactams induce a PAE of 1.2-4.5 hours to *S.aureus*, but no PAE to gram-negative bacteria and *S.pneumoniae* were observed. These results are consistent with in vitro results with the exception of penicillin in relation to *S.pneumoniae*.[95]

The most important clinical application of the PAE as a pharmacodynamic parameter is in defining optimal dosing schedules for β-lactams. The PAE is crucial in determining the optimal time above the MIC for any given drug-pathogen combination. The existence of a PAE implies that the time above the MIC can be less than 100% of the dosing interval and, in fact, the PAE is the theoretical rationale for the intermittent dosing of β-lactams.

3.2.2 Protein binding

The effect of protein binding continues to be a topic of debate even as more is learned about the pharmacodynamics of β-lactams. It is recognized that protein binding is a rapid process that produces a reversible interaction between antibiotic and protein, principally albumin. A constant equilibrium exists between the total (T) drug concentration and free/unbound (F) and protein bound (DB) fractions: $T \leftrightarrow F+DB$. It is accepted that only the free drug is able to diffuse from the blood stream to the site of infection and subsequently into the bacteria. This concept is important for the time-dependent antibiotics that rely on maintaining unbound serum concentrations in excess of the MIC for a prolonged period of time. For highly protein bound drugs, failures may be predicted as free-drug concentrations drop below the MIC.[92]

The in vitro and in vivo reduction in cephalosporin activity is less predictable when cephalosporins are tested against gram-negative organisms. Leggett and Craig found that the ceftriazone, cefoperazone, moxalactam and ceftizoxime MICs for *E.coli* and *K.pneumoniae* in human serum ultratfiltrate were less than predicted by simply examining the protein binding alone. The same effect was not observed with *S.aureus* or *P.aeruginosa*. A disproportionate rise in the MIC was observed when the antibiotics were place into 25%, 50%, and 95% serum.[93] The hypothesis for this observation was that the serum contained products that enhanced the killing of gram-negative bacilli. In essence, a protein found in the serum effectively lowered the MIC when these cephalosporins were exposed to Enterobacteriaceae. This finding may explain why ceftriaxone (95% protein bound) provides activity at or slightly below its MIC, even though failure would be predicted.

Studies evaluating the effect of protein binding and clinical outcome in humans are difficult to perform. Clinical data in human to show that protein binding can

alter the outcome of an infection were presented in a case report by Chambers et al.[94] These workers reported three therapeutic failures with cefonicid (95% protein bound) used once daily for the treatment of *S.aureus* endocarditis. It was suggested that free drug concentrations exceeded 150 μg/ml, but the serum bactericidal titer (SBT) was < 1:8, Successful outcomes are predicted when the SBTs are ≥1:8. Another consequence of the high protein binding was the MIC difference of cefonicid in broth versus serum. When the organisms were originally tested in broth, the mean for all patients was 4.6 μg/ml, whereas use of 50% serum as the diluent resulted in a six-fold increase (27.9 μg/ml). As noted previously, protein binding, particularly in situations with highly bound cephalosporins against gram-positive bacteria, will result in an increase of the MIC. From these data, it appears that this regimen allowed free-drug concentrations to remain above the MIC for only a very short time. The role of protein binding may affect the pharmacodynamics of specific antibiotic-bacterium combinations.

3.3 Research studies

When the T>MIC exceeds 40 to 50% of the dosing interval, clinical and bacteriological efficacy theoretically approaches > 90%. However, a few qualifying statements need to be made. Several studies conducted in animal models showed only the static effects on bacteria and not bactericidal. In a murine thigh model of infection,[14] it was noted that the T>MIC required to achieve the same outcome was consistenly higher for cephalosporins (35-53% of dosing interval) than for penicillin (29-34% of the dosing interval) or carbapenems (20-26% of the dosing interval). These differences correlated with differences in rates of bacterial killing, which were greater in association with carbapenems (i.e., the T>MIC required for an equivalent effect was lower for carbapenems). In addition, the degree of antibacterial protein binding can alter the T>MIC. Ceftriaxone (80% protein bound) requires a larger T>MIC than does cefotaxime (10-20% protein bound), when calculations are based on the total drug concentration, supporting the concept that free drug is the biologically relevant parameter. According to the results of in vitro and in vivo animal studies show that β-lactams require varying fT>MIC. For instance, carbapenems require 20% and 40% of fT>MIC for bacteriostatic and bactericidal activity, whereas cephalosporins require 35-40% for bacteriostasis and 60-70% for maximum bactericidal activity. Penicillins generally inhibit bacterial growth at 30% fT>MIC and achieve bactericidal exposures at 50% fT>MIC.[14,15]

For human data, the study in patients with otitis media and acute maxillary sinusitis, it appears that a T>MIC of approximately 40% for penicillins and 50% for cephalosporins achieves high bacteriological eradication rates.[95] More recently, the

pharmacodynamics of cefepime (plus aminoglycoside) was determined in 20 patients with gram-negative infections. These investigators observed a strong link between microbiological success and T>MIC. Success was 89% when the total T>MIC was 100% compared with 0% when T>MIC was <100% (p=0.032). Utilizing classification and regression tree analysis (CART), a minimum concentration (Cmin)/MIC >4.3 was independently predictive of response. These investigators determined that in order to achieve a probability of 80-90% microbiological success, serum concentrations would need to exceed 4.3 X MIC for 83% and 95% of the dose administration interval, respectively.[96] Similarly, Lee et al.[97] evaluated clinical cure in patients receiving cefepime monotherapy against ESBL and non-ESBL strains of *E.coli* and *Klebsiella spp*. Eradication was 80% when the total drug T>MIC was 100% compared with 0% when T>MIC was <100% (p=0.025). CART identified that Cmin/MIC was more predictive of eradication than T>MIC. Regardless of ESBL production, all pathogens were eradicated when Cmin/MIC > 8.9 and only 33.3% were eradicated when Cmin/MIC $\leq$ 8.9 (p=0.009). In a recent study, Tam et al. described,[98] pharmacodynamic relationships for cefepime in the treatment of patients with gram-negative infections. The results supported previous findings that the bactericidal activity of cefepime was optimal at concentrations approximately four times the MIC against various gram-negative organisms. It appears that there may be situations in which T>MIC is not the only important parameter to contribute to clinical outcome, and that some magnitude of concentration in excess of the MIC may be required to optimize therapy. With further understanding of bacterial populations, it is becoming increasingly evident that both the magnitude of serum concentration achieved and T>MIC are important to the efficacy of β-lactam antibiotics. In instances in which there was testing of very sensitive organisms or in which the investigators were using a low bacterial inoculum, concentration independence has been demonstrated and T>MIC has been identified as the significant pharmacokinetic parameter. However, in studies utilizing more resistant organisms or larger inoculum sizes, there is a demonstrated concentration-dependent effect, which is likely related to the distribution frequency of resistant subpopulations, and subsequent derepression or selection of resistant organisms during therapy.

The pharmacodynamic concepts for β-lactam antibiotics also influenced the mode of intravenous drug administration. Several studies reevaluated the efficacy and safety of continuous infusion of β-lactam agents. Some studies demonstrated that the total daily dose is lower with continuous infusion than with intermittent intravenous therapy. Whereas this mode administration allows for optimization of T>MIC, its application at the bedside is limited

to severe life-threatening infections (e.g., *P.aeruginosa*), and outpatient intravenous therapy. In addition, several questions remain unanswered, including the necessity for a loading dose, the ideal ratio between unbound drug concentration and MIC value, the need to monitor drug concentrations and pharmacoeconomic advantages.[99]

3.4 β-lactam and β-lactamase inhibitor combinations

Strategies for the optimal dosing β-lactams and β-lactamase inhibitors (clavulanate, sulbactam and tazobactam) based on pharmacodynamic principle have not been established or even extensively studied. In addition to the pharmacodynamic issues that relate to the individual β-lactam, several additional pharmacodynamic questions arise in relation to combination drugs.

Two pharmcodynamic studies have addressed the issue of sequential dosing. In vitro, the sequential dosing of tazobactam followed by piperacillin does not enhance the bactericidal activity of piperacillin.[100] Similarly, in vivo, as studied in *E.coli* bacteremia in mice, the pharmacodynamics of ampicillin-sulbactam does not depend on whether sulbactam is dosed sequentially or simultaneously with ampicillin.[101]

For the optimal T>MIC, is complicated for β-lactam-β-lactamase inhibitors combinations because the turnover rate of both the enzyme and the inhibitor will affect the inactivation of β-lactamase. The amount and type of enzyme produced by the bacteria have a marked effect on the pharmacodynamic of the inhibitor. Current dosing regimens provide concentrations of inhibitor that exceed the in vitro susceptibility breakpoint for only 2-3 hours, not the entire dosing interval. One explanation from the clinical efficacy of these drugs may relate to a post-β-lactamase inhibition effect. In an adaptation of the model used to determine post-antibiotic effect, the effect of tazobactam was evaluated in β-lactamase-producing strains of *E.coli*. Preincubation of bacteria with tazobactam and piperacillin resulted in piperacillin-induced killing during a second exposure to piperacillin alone. Bacteria not initially exposed to tazobactam were not killed by piperacillin during the second exposure. Similarly, other investigators have reported a post β-lactamase inhibitor effect in which regrowth of amoxicillin-resistant.[102]

3.5 The application of Monte Carlo simulation in β-lactams antibiotics

Monte Carlo simulation has been used by several investigators for various functions. It has been used to determine the pharmacodynamic profile of both approved and study antimicrobials, to optimize antimicrobial dosing against a known or suspected MIC

distribution of organism(s), to establish the optimal dosage for a new compound, and to estimate the ability of antimicrobials to penetrate the site of infection. Monte Carlo simulation is considered by the Clinical Laboratory Standards Institute (CLSI) to establish antibiotic susceptibility breakpoints for new antibiotics and assess the validity of existing breakpoints for United States Food and Drug Administration-approved antibiotics.

Piperacillin – tazobactam

Based on a Monte Carlo simulation that used pharmacokinetic data from healthy male volunteers, piperacillin-tazobactam 3.375 g infused over a 4-hour period every 8 hours (extended infusion) was identified as an alterative to the traditional dosing regimen of piperacillin-tazobactam 3.375 infused over 30 minutes every 4 or 6 hours. The pharmacodynamic end point selected for this simulation, 50% $fT>MIC$, correlated with maximum bactericidal activity for penicillins. Monte Carlo simulation revealed that the PTA for extended infusion piperacillin-tazobactam was 92% at 16 mg/L and 100% for lower MIC. In contrast, with a 30-minute infusion of piperacillin-tazobactam every 4 hours, the PTA was greater than 90% only for MIC values of 8 mg/L or less; with higher MICs, the PTA was below 90%. For a 30-minute infusion of piperacillin every 6 hours, The PTA was more than 90% only for an MIC value of 1 mg/L. For MIC of 32 mg/L or greater, no regimen was optimal.[103]

The extended-infusion dosing strategy for piperacillin-tazobactam offered two benefits in addition to its superior pharmacodynamic profile. First, this regimen allowed 4 hours within each 8-hour dosing interval could be administered in which other agents could be administered through the same intravenous line. Second, it provided an economic benefit by reducing the amount of dose/patient/day by one or three. Reducing the total daily dose by 25-50% represented a potential savings of approximately $ 68,750-137,000 in direct drug acquisition costs/year.[104]

Meropenem

A Monte Carlo simulation using pharmacokinetic data from healthy volunteers[105] showed that extended infusion meropenem provided more probabilities of target attainment than either conventional meropenem dosing regimens or imipenem 500 mg every 6hours administered as a 1 hour infusion. Using the global Meropenem Yearly Susceptibility Testing Information Collection (MYSTIC) surveillance data as the measure of MIC distribution and calculated the PTA for various nosocomial pathogens. Meropenem 500 mg every 8 hours (1-and 3-hr infusion) had excellent comverage except *P.aeruginosa* and *Acinetobacter* spp. For these pathogens, meropenem 1000 mg every 8 hours, administered by 3-hour infusion, provided higher PTA. Based on these results; conventional meropenem dosing was changed to meropenem 500 mg every 8 hours as a 3-hour infusion was reserved for suspected

infection with either *P.aeruginosa* or *Acinetobacter* spp. For situations in which these pathogens were not suspected, 1-hours infusion of meropenem 500 mg every 8 hours is recommended because this regimen had a similar PTA and required less administration time than the alternative regimen.

Cefepime

Using 67% *f*T>MIC as the pharmacodynamic target. Cefepime 1000 mg every 6 hours as a 0.5-hour infusion was identified as an alternative to conventional cefepime dosing. Multiple extended-infusion regimens were evaluated, and all cefepime dosing provide high probability of target attainment against the range of MICs deemed susceptible by the CLSI [106]

Cefepime 1000 mg every 6 hours as a 0.5-hour infusion was adopted as the new dosing strategy for several reasons. First, this regiment provided more PTA than conventional cefepime dosing (1000 mg every 12 hrs as a 0.5 hrs infusion). Second, the new regimen (cefepime 1000 mg intravenously every 6 hrs) has similar probability of target attainment profile as maximal cefepime dosing. (2000 mg every 8 hrs as a 0.5-hr infusion), but it optimized *f*T>MIC while using a smaller amount of drug (2g/day less). Given that cefepime costs approximately $12.5/g, the projected drug acquisitions cost savings were considerable. Finally, cefepime 1000 mg intravenously every 6 hours achieved the targeted *f*T>MIC with less administration time/day than prolonged infusion.