Alexander A. Vinks · Hartmut Derendorf
Johan W. Mouton *Editors*

Fundamentals of Antimicrobial Pharmacokinetics and Pharmacodynamics

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Contents

Part II Clinically Oriented Chapters

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Part I
Basic Concepts and Principles

Chapter 1
Introduction to Pharmacodynamics

William A. Craig

Abstract Since the early appreciation of differences in the time course of antimicrobial activity, much has been learned about the pharmacodynamics of antimicrobials. Specific PK/PD indices have been identified which are of major importance for efficacy and for the prevention of the emergence of resistance. Of major importance, the magnitudes of these PK/PD indices for efficacy have been shown to be very similar in animal infection models and human infections. Modeling has also identified that there are few differences in the index magnitude with different dosing intervals, among drugs within the same antimicrobial class (providing free drug concentrations are used), with different infection sites (except occasionally for pneumonia), and among susceptible and resistant strains of the same type of bacteria. Addition studies have shown that the magnitude of indices can increase significantly with a higher inoculum for *S. aureus* and that neutrophils have a minor enhancing effect on antimicrobial activity against Enterobacteriaceae but a more variable enhancing effect on activity against *S. pneumonia* for different antimicrobials. Pharmacodynamic modeling has many applications including establishing new optimal dosing regimens, developing new antimicrobials and formulations, determining susceptibility breakpoints, providing guidelines for empiric therapy, and formulary development.

Keywords Pharmacodynamics • Modeling • PK/PD indices • Protein binding • Animal models • Neutrophil activity • Inoculum size • Susceptibility testing

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Introduction

Antimicrobial pharmacodynamics deals with the relationship between measures of drug exposure and the efficacy and toxicity of antimicrobial agents. Since the early days of penicillin, researchers have been interested in determining which pharmacokinetic parameter is most important in determining microbiologic and clinical efficacy. For example, bacterial killing of staphylococci by penicillin was much different than by streptomycin (Garrod 1948). The rate of killing by penicillin was not dependent on the height of the drug concentration, while streptomycin demonstrated enhanced killing at higher concentrations. Studies in mice-infection models suggested that the duration of drug exposure was the most important parameter determining in vivo therapeutic efficacy of penicillin (Eagle et al. 1950). Interest in antimicrobial pharmacodynamics increased in the 1960s and 1970s when infections due to *Pseudomonas aeruginosa* with high MICs to multiple drugs appeared with increasing frequency (Rolinson 1973). This interest in antimicrobial pharmacodynamics has been further enhanced by the emergence of antimicrobial resistance to many drugs during the last 15–20 years.

Time Course of Antimicrobial Activity

A major determinant of the time course of antimicrobial activity is whether the drug exhibits bactericidal activity and whether the killing is enhanced by increasing concentrations or by longer exposure times. The second major determinant is whether the drug exhibits persistent inhibition of growth that lasts after the drug exposure. There are numerous in vitro persistent effects described in the literature that usually act together in the in vivo situation. The in vitro postantibiotic effect (PAE) describes the extent of continuing retardation in organism growth when the drug is suddenly removed by repeated washing, dilution, filtration, or inactivation (McDonald et al. 1977; Bundtzen et al. 1981). The postantibiotic sub-MIC effect (PA-SME) identifies additional prolongation in regrowth that results from sub-MIC drug concentrations (Cars and Odenholt-Tornqvist 1993). The postantibiotic leukocyte enhancement (PALE) identifies growth retardation that occurs when organisms in the postantibiotic state of growth are exposed to leukocytes (McDonald et al. 1981).

As stated above, these various in vitro persistent effects act together in describing the in vivo activity of antimicrobials. By injecting penicillinase intravenously in neutropenic mice when drug levels of piperacillin or aspoxicillin were expected to drop below the MIC, Oshida et al. (1990) were able to show that sub-MIC concentrations accounted for a little less than half of the 3.3 and 5.2 h in vivo postantibiotic effect observed with both drugs, respectively, against *Staphylococcus aureus*. Increasing the dose (and AUC) of amikacin sixfold increased the duration of the in vivo postantibiotic effect with *Klebsiella pneumoniae* in neutropenic mice from 3.4 to 7.4 h, while the duration of sub-MIC values was less than an hour and virtually the same for both doses (Craig et al. 1991). However, prolonging the half-life of

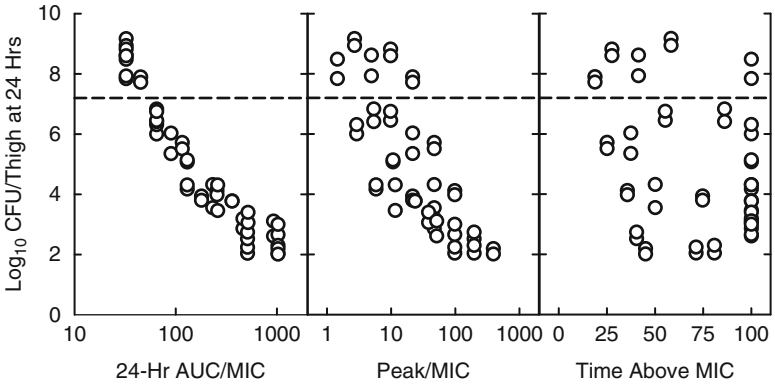

Fig. 1.1 Relationship between three PK/PD indices for total drug of levofloxacin and the $\log_{10}$ CFU/thigh at 24 h for *Streptococcus pneumoniae* ATCC 10813 in the thighs of neutropenic mice. Reproduced with permission from Andes and Craig (2002)

amikacin from 18 to 110 min by inducing renal impairment also enhanced the AUC about sixfold, but the longer duration of sub-MIC concentrations increased the in vivo postantibiotic effect from 7.4 to 12.2 h. The role of leukocytes on the in vivo PAE has also been assessed. Studies with similar doses of gentamicin against the same strain of *K. pneumoniae* have reported in vivo PAEs of 7.8, 12.0, and 16.5 h in neutropenic, normal, and granulocytic mice, respectively (Shimizu et al. 1989).

Patterns of Antimicrobial Activity

Three major patterns of antimicrobial activity have been observed. The first applies to antimicrobials with concentration-dependent killing along with prolonged persistent effects. This pattern is observed with aminoglycosides, fluoroquinolones, polymyxins, daptomycin, and some of the new glycopeptides, such as telavancin and oritavancin, which also exhibit an additional membrane effect mechanism of action. One would predict that the ratio of the AUC and peak concentration to the MIC would be the primary PK/PD indices correlating with antimicrobial efficacy. Done-fractionation studies in animal models of infection in which five or six total doses are divided into many smaller doses given at different dosing frequencies have been useful in reducing the interdependence among the PK/PD indices and confirming which PK/PD index is most important for efficacy. The relationship of all the PK/PD indices based on total drug concentrations (protein binding in mice 15 %) to efficacy of levofloxacin against *Streptococcus pneumoniae* in the thighs of neutropenic mice are shown in Fig. 1.1 (Andes and Craig 2002). The 24-h AUC/MIC showed the best correlation for efficacy followed by the peak/MIC ratio. The time above MIC looked more like a scattergram.

The second pattern of antimicrobial activity is the exact opposite of the first pattern with concentration-independent killing and no or very short persistent effects. This pattern is characteristic of all of the ß-lactam antibiotics, such as penicillins,

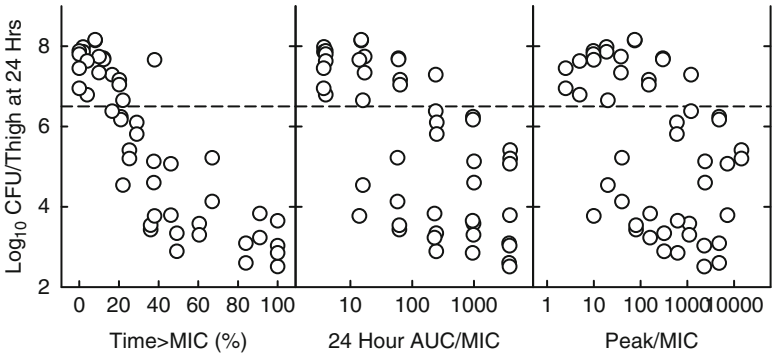

Fig. 1.2 Relationship between three PK/PD indices for total drug of imipenem and the $\log_{10}$ CFU/thigh over 24 h for *Pseudomonas aeruginosa* ATCC 27853 in the thighs of neutropenic mice

cephalosporins, carbapenems, and monobactams. With this pattern, one would predict that the duration of time that active antibiotic concentrations exceeded the MIC would be the important PK/PD index for efficacy. Figure 1.2 demonstrates the relationships among the various PK/PD indices for total drug concentration of imipenem, a carbapenem ß-lactam antibiotic with protein binding <5 % in mice, against a standard strain of *Pseudomonas aeruginosa* in the thighs of neutropenic mice. The percentage of the dosing interval that concentrations exceeded the MIC showed the best correlation with organism growth and killing, while the relationships with AUC/MIC and peak/MIC looked more like scattergrams.

The third pattern of antimicrobial activity also exhibits concentration-independent killing but these antimicrobials induce prolonged persistent effects. This pattern is observed with a large number of antimicrobials including the tetracyclines, tigecycline, macrolides, azithromycin, clindamycin, linezolid and other oxazolidinones, chloramphenicol, trimethoprim, sulfonamides, vancomycin, and dalbavancin. Because the prolonged persistent effects will protect against regrowth when active drug concentration fall below the MIC, one would predict that the amount of drug or the AUC/MIC would be the important PK/PD index for these drugs. Figure 1.3 illustrates that relationship between the change in efficacy from the start of therapy and the various PK/PD indices based on total drug concentrations for vancomycin (protein binding 13 % in mice) (Rybak 2006). The best correlation for efficacy was seen with 24-h AUC/MIC index. Peak/MIC and time above MIC showed much more variation in efficacy at different magnitudes of the index.

Magnitude of Index Required for Efficacy

Once the important PK/PD index driving efficacy is identified, the next piece of information needed is what magnitude of the index is required for antimicrobial efficacy. A large number of animal studies on the efficacy of ß-lactams against

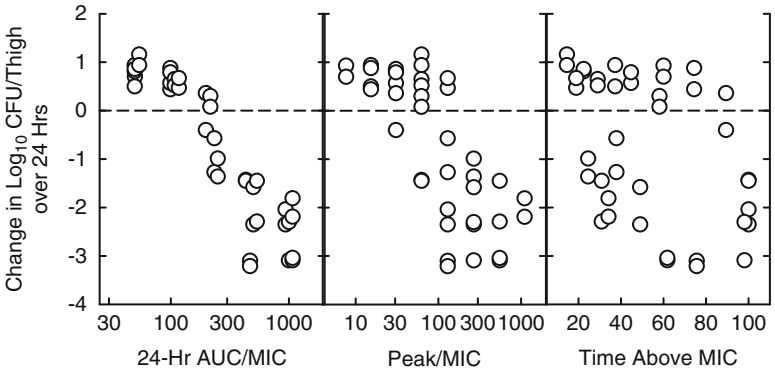

Fig. 1.3 Relationship between three PK/PD indices for total drug of vancomycin and the change in $\log_{10}$ CFU/thigh over 24 h for *Staphylococcus aureus* ATCC 25923 in the thighs of neutropenic mice. Redrawn from data in Rybak (2006)

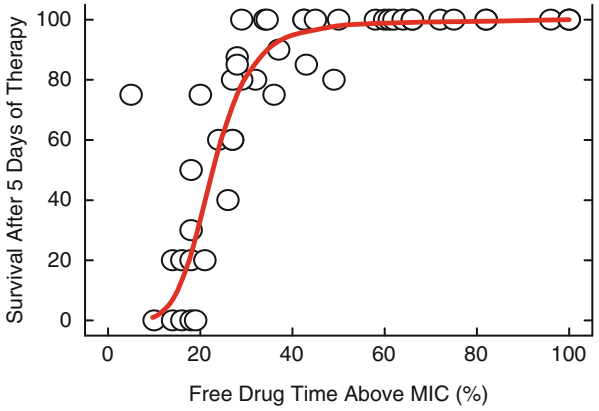

Fig. 1.4 Relationship between survival in neutropenic mice infected with strains of *Streptococcus pneumoniae* and time above MIC for various penicillins and cephalosporins. Redrawn from data in Andes and Craig (2002)

S. pneumoniae and fluoroquinolones against Enterobacteriaceae and *P. aeruginosa* have evaluated different index magnitudes in various infection models using survival as the endpoint. The infections included pneumonia, peritonitis, bacteremia, and thigh-infection models. Untreated or saline-treated controls had 80–100 % mortality by the end of each study. Figure 1.4 shows the relationship between various free drug time above MIC values for penicillins and cephalosporins versus survival of mice with *S. pneumoniae* infections (Andes and Craig 2000; Nicolau et al. 2000). Ninety percent (90 %) or higher survival was observed when time above MIC was 35 % or higher. Figure 1.5 illustrates the relationship between 24-h AUC/MIC values for multiple fluoroquinolones and survival of mice, rats, and guinea pigs infected with Enterobacteriaceae or *P. aeruginosa* (Andes and Craig 2002; Craig and Dalhoff 1998). This time 90 % or higher survival was observed when the 24-h AUC/MIC value was 105 or higher. This value is equivalent to averaging a little over four times the MIC for 24 h. Survival was only 50 % when the 24-h AUC/MIC value was 41.

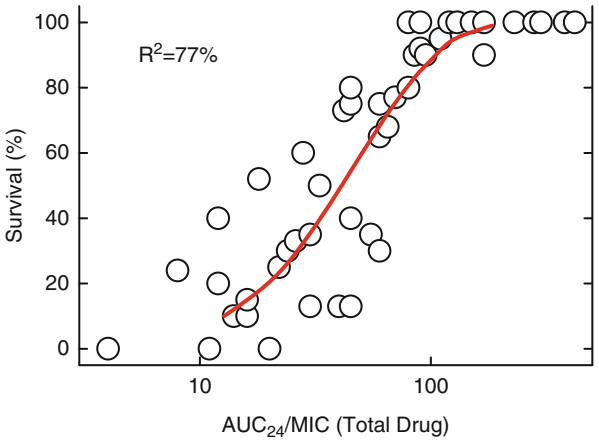

Fig. 1.5 Relationship between survival in various neutropenic animals infected with gram-negative bacilli and 24-h AUC/MIC for multiple fluoroquinolones. Redrawn from data in Andes and Craig (2002)

Since most PK/PD studies are initially performed in animal or in vitro infection models, it would be important to know if the magnitudes observed in these models are also predictive of the magnitudes required for clinical efficacy in patients. One would expect a good correlation since the receptors for activity are in the organism and not in the animal or human. Studies in otitis media employing a double tap typanocentesis technique have shown that greater than 40 % time above MIC results in 85–100 % bacterial eradication (Craig and Andes 1996). In patients with severe gram-negative infections treated with ciprofloxacin, efficacy was greater than 80 % in those with a 24-h AUC/MIC value of 125 or greater (Forrest et al. 1993). Both of these studies are similar to the results described above in various animal models (Figs. 1.4 and 1.5, respectively). Many more correlations have been made for other antimicrobials, and the results in animal models have been very similar to results derived from human clinical trials (Ambrose et al. 2007).

Factors Affecting the Magnitude of the Index Required for Efficacy

Dosing Regimen

Many animal infection studies have demonstrated that the magnitude of the PK/PD indices usually does not change with different dosing intervals. Those that have shown increasing magnitudes at longer dosing interval are antimicrobials with very rapid half-lives in mice (less than 30 min). For example, the magnitude of the 24-h AUC/MIC for amikacin was similar for 1-, 3-, and 6-h dosing intervals, but got increasing larger as the dosing interval went up to 12- and 24-h dosing frequencies (Craig et al. 1991). However, when the half-life of amikacin was increased from 18 to 110 min by drug-induced renal impairment, the magnitude of the 24-h AUC/MIC

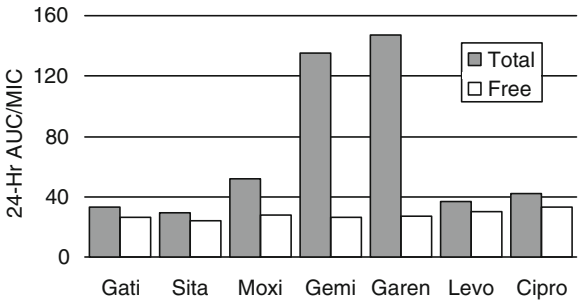

Fig. 1.6 The 24-AUC/MIC values for total and free drug required for stasis with seven fluoroquinolones. Redrawn from graph in Ambrose et al. (2003)

for gentamicin with 6-, 12-, and 24-h dosing intervals were virtually identical. Erythromycin and clindamycin are other antimicrobials with rapid half-lives in mice that make only dosing intervals up to 6 h valid for magnitude measurements.

Protein Binding

Since only free, unbound drug has antimicrobial activity, protein binding should have a major effect on the magnitude of the index required for efficacy. Many years ago Kunin demonstrated that the in vitro activity of different ß-lactam antibiotics in human serum was virtually identical to the fraction of drug that was free and not bound to serum proteins (Kunin 1966). Studies in animal sera have generally shown lower percentages for protein binding than in human sera. In the murine thigh model with six cephalosporins against a standard strain of *Klebsiella pneumoniae,* studies have demonstrated that the magnitude of the percentage of time that serum concentrations need to exceed the MIC to produce stasis can vary from 34 to 63 % (Craig 2003). However, when free drug concentrations were used, the variation was only from 31 to 38 %. Figure 1.6 shows the variation in the magnitude of the 24-h AUC/MIC required for stasis against a standard strain of *S. pneumoniae* in the thighs of neutropenic mice for seven fluoroquinolones (Ambrose et al. 2003). For total drug the variation in the 24-h AUC/MIC for the seven drugs was approximately fivefold. For free drug the variation was minimal and no more than 1.2-fold. Thus, when examining the magnitudes of the index required for efficacy for different antimicrobials of the same class, the free drug concentrations should be used for comparison.

Antibiotic Class

Different classes of ß-lactams can affect the magnitude of the PK/PD index observed with streptococci and gram-negative bacilli. Since these organisms do not exhibit persistent effects, any difference in the magnitude of the PK/PD index would have to reflect variations in the rate of bacterial killing. Cephalosporins kill

bacteria a little slower than penicillins, and penicillins kill slower than the carbapenems. The difference in rate of killing is most marked for carbapenems with strains of *S. pneumoniae* that require only 10–20 % time above MIC to induce bacterial stasis (Craig 2003). The monobactams, such as aztreonam, behave similar to the cephalosporins in terms of rate of killing (Craig et al. 1993).

Organism and Strains

There are few organism or strain differences in the magnitude required for stasis observed within each antimicrobial class in most neutropenic animal models. Penicillin and cephalosporin antibiotics do induce in vivo postantibiotic effects of 3–5 h with staphylococci that are not observed with streptococci or gram-negative bacilli (Craig and Gudmundsson 1996). This results in staphylococci requiring less time for active drug concentrations of these ß-lactams to exceed the MIC to produce efficacy compared with streptococci and gram-negative bacilli (Craig 1995). On the other hand, carbapenems show minimal differences in the time above MIC required for stasis with staphylococci and *P. aeruginosa* as both organisms induce modest persistent effects (Craig and Gudmundsson 1996). For other antimicrobials, such as the fluoroquinolones and tetracyclines, there are only minimal differences among streptococci, staphylococci, and gram-negative bacilli in the magnitudes of the 24-h AUC/MIC required for stasis. Still differences in the magnitude for 90 and 99 % killing (i.e., 1 and 2 $\log_{10}$ kill) can occur among these organisms because of variations in the rate of killing.

Presence of Drug Resistance Mechanisms

The same index magnitude required for susceptible bacterial strain also appears to apply to those expressing resistance mechanisms. For multiple β-lactams, the percentage of time above the MIC for free drug in the neutropenic murine thigh model was very similar for penicillin-susceptible, penicillin-intermediate, and penicillin-resistant strains of *S. pneumoniae* (Craig 2007). This was observed with two different penicillins, three different cephalosporins, and three different carbapenems. As the MIC to the various β-lactams increased for the penicillin-intermediate and -resistant strains, higher doses of drug were required for efficacy, but the time above MIC was still similar to that observed with susceptible strains. In the same neutropenic murine thigh model, gemifloxacin, a fluoroquinolone with enhanced activity against *S. pneumoniae*, exhibited the same 24-h AUC/MIC values for susceptible pneumococcal isolates and resistant strains with various ParC, ParE, and/or GyrA mutations (Andes and Craig 1999). However, strains with efflux as the mechanism of resistance were about five times more susceptible in vivo than fluoroquinolones-susceptible or mutation-resistant strains.

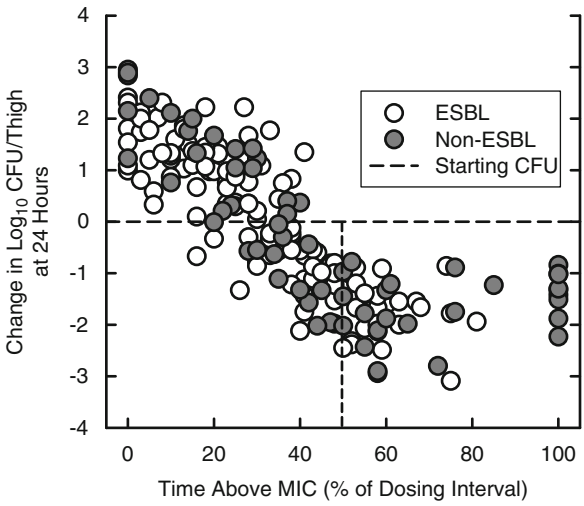

Fig. 1.7 Relationship between the percent time above MIC for free drug of 4 extended-spectrum cephalosporins and the change in the $\log_{10}$ CFU/thigh at 24 h for organisms with and without ESBLs. Reproduced with permission from Andes and Craig (2005a)

Studies with several β-lactam antibiotics against gram-negative bacilli with or without extended spectrum β-lactamases (ESBLs) or carbapenemases have demonstrated that the same time above MIC is required for both groups of organisms. Figure 1.7 shows the relationship between free drug time above MIC and the change in $\log_{10}$ colony-forming units (CFUs) over 24 h in the neutropenic murine thigh-infection model when Enterobacteriaceae with or without ESBLs were exposed to different doses of ceftazidime, cefepime, cefotaxime, and ceftriaxone administered every 6 h (Andes and Craig 2005a). The time above MIC observed for susceptible strains without ESBLs were identical to the values seen with the largely resistant organisms with ESBLs. The same finding has been reported by another investigator using both low and high inocula in a similar mouse model with cefepime alone (Maglio et al. 2004). More recently, the activity of doripenem, meropenem, and imipenem against susceptible Enterobacteriaceae and strains of *Klebsiella* species containing carbapenemases were compared using the neutropenic murine thigh-infection model (Craig et al. 2008). Again the time above MIC required for efficacy was similar for both groups of organisms.

Immunity Status

As most in vivo pharmacodynamic studies are performed in neutropenic animals, the presence of neutrophils in these models of infection can reduce the magnitude of the PK/PD index required for efficacy. However, the impact of neutrophils on the magnitude of the PK/PD index varies for different organisms and for different antimicrobials. Using a strain of *K. pneumoniae* that grows well in both neutropenic and normal mice, the presence of neutrophils had a small effect and dropped the time above MIC for various penicillins, cephalosporins, and carbapenems only about 5–8 percentage points (Andes and Craig 2002; Craig et al. 1990). Similarly,

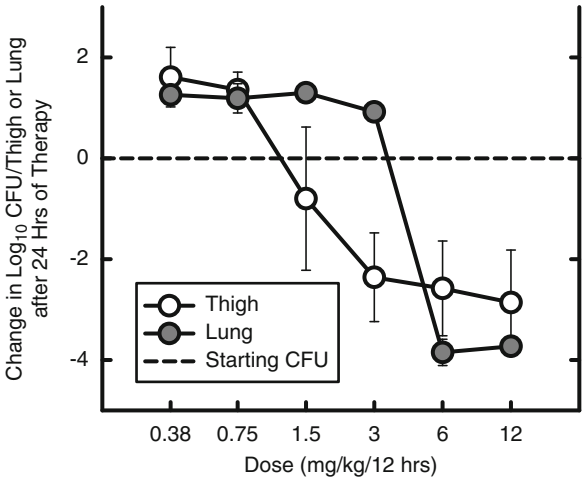

Fig. 1.8 Relationship between the change in $\log_{10}$ CFU after 24 h for *Streptococcus pneumoniae* ATCC 10813 in the thigh and lung of neutropenic mice and the magnitude of the doses of vancomycin given every 12 h

the presence of neutrophils reduced the 24-h AUC/MIC values for aminoglycosides and fluoroquinolones by 1.5 to 2-fold.

Neutrophils had a more varied effect with *S. pneumoniae* on enhancing activity of antimicrobials. Little enhancement was observed with β-lactams, a two- to four-fold enhancement was observed with tetracyclines and macrolides, and a five- to sixfold enhancement was observed with clindamycin and fluoroquinolones (Andes and Craig 2002; Craig and Andes 2000; Christianson et al. 2002).

Site of Infection

There are small differences in the magnitude of the PK/PD index in septicemia, peritonitis, and thigh-infection models (Andes and Craig 2002). The free drug concentrations in peritoneal fluid and interstitial fluid of thighs are similar to those in serum. However, pneumonia-infection models can produce enhanced or reduced activity compared to other sites depending on the drug's penetration into epithelial lining fluid (ELF). Figure 1.8 compares the activity of vancomycin against a standard strain of *S. pneumoniae* in the thigh and lung of the same mice (Craig and Andes 2004). Vancomycin was about threefold less active in the lung than in the thigh. Macrolides and aminoglycosides have tended to be more active in the lung than in the thigh (Maglio et al. 2003; Leggett et al. 1989).

Inoculum Size

Significant increases in the magnitude of the PK/PD for efficacy with most antimicrobials have not been observed with higher inocula of *S. pneumoniae* and various gram-negative bacilli (Andes and Craig 2002; Maglio et al. 2004). On the

other hand, sizeable increases in the 24-h AUC/MIC values required for stasis have been observed for daptomycin, linezolid, and vancomycin with *Staphylococcus aureus* when the starting inoculum is increased from 10^5 to 10^7 CFU per thigh (Lee et al. 2007). About fourfold increases in the magnitude were observed with daptomycin and linezolid, and a sevenfold increase was seen with vancomycin. In vitro models have not shown similar increases in the amount of these antibiotics required for efficacy. This in vivo phenomenon may be related to an old observation that staphylococci growing in vivo look like organisms growing on a surface (Lorian et al. 1985).

Drug Combinations

There is very limited information on the impact of drug combinations on the magnitude of the PK/PD index driving efficacy. It was suggested by some investigators that the magnitudes of the 24-h AUC/MIC for each individual drug could be added to estimate the magnitude of the combination (Thomas et al. 1998). A study in the neutropenic murine thigh model with *P. aeruginosa* compared the activity of various combinations of ceftazidime, netilmicin, and ciprofloxacin (Mouton et al. 1999). Adding up the 24-h AUC/MIC values of ceftazidime plus netilmicin and ceftazidime plus ciprofloxacin gave a poor prediction of the combined drug activity. Time above MIC for ceftazidime and the 24-h AUC/MIC for netilmicin or ciprofloxacin were much better predictors of the combined drug activity. However, adding up the 24-h AUC/MIC values for netilmicin and ciprofloxacin were good predictors of combined drug activity since the AUC/MIC is the major index for both drugs. Thus, it appeared that the magnitude of the PK/PD index when used alone was also important when used in drug combinations.

PK/PD Targets for Resistance Suppression

The increasing resistance to antimicrobials has stimulated researchers to identify the magnitude of the PK/PD index that is most important in preventing the emergence of resistant mutants. Some researchers feel that the emergence of resistance in related to the intensity of the dose. At very low, ineffective doses, mutants do not emerge, while the susceptible population continues to grow. As the doses increase, the susceptible population is inhibited or killed and resistant mutants start to emerge. At even higher doses, the susceptible population and any prior existing mutants are both inhibited or killed. The overall picture on the emergence of resistant mutants based on the intensity of the dose is represented by an inverted "U." For example, no resistant mutants of a fluoroquinolones susceptible MRSA were observed when exposed to broth alone, mutant selection was maximal when exposed to garenoxacin at an AUC/MIC value of 35, and resistance was completely repressed when exposed to garenoxacin at an AUC/MIC value of 143 (Tam et al. 2007a).

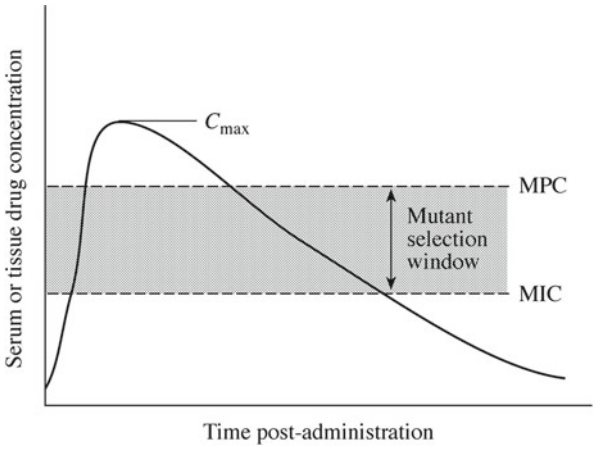

Fig. 1.9 Depiction of the mutant selection window

Another concept involved in resistance suppression was the mutant prevention concentration or MPC (Blondeau et al. 2001). That is the concentration of drug that prevents the emergence of resistant mutants on agar at a high 10^{10} inoculum of bacteria. For many antimicrobials the MPC is 4–8 times higher than the MIC for *S. pneumoniae* and 16–64 times higher for *S. aureus* and *E.* coli (Blondeau et al. 2001; Drlica 2003). For a few drugs such as rifampin, the MPC is more than 500 times higher than the MIC. The mutant selection window is defined as the difference between the MIC and the MPC as depicted in Fig. 1.9 (Firsov et al. 2006). Drug concentrations within the mutant selection window would be expected to select resistant mutants, while concentration above the mutant selection window would prevent the selection of resistant mutants. Studies in a dynamic in vitro model of daily dosing of daptomycin and twice daily dosing of vancomycin for 6 days have shown no selection of resistant mutants when drug concentrations were below the MIC or above the MPC (Firsov et al. 2006). However, resistant mutants were selected when at least 30 % of the drug concentrations of each drug were in the mutant selection window. The mutant selection window is not necessarily different than the inverted "U" for dosing intensity. When one looks at 24-h AUC/MIC exposures in the dynamic in vitro model, resistance was not observed at values of 13–16 and 216–256, but was seen at 24-h AUC/MIC values of 32–64. Some studies have suggested that the 24-h AUC/MPC varies less with strains than the 24-h AUC/MIC. In animal models infected with *S. aureus* and *P. aeruginosa*, enrichment of resistance was observed with fluoroquinolones at 24-AUC/MIC values of 25–100 and 24-h AUC/MPC values of 3–24 (Jumbe et al. 2003; Cui et al. 2006). Resistance was suppressed at 24-h AUC/MIC values >150 and 24-h AUC/MPC values >25 for the same fluoroquinolones.

For β-lactams, dose fractionation studies have demonstrated that time above MIC was the important index with magnitudes of 50–100 % for preventing the emergence of resistance (Louie et al. 2010). A trough level of 4 mg/L was effective in preventing the emergence of resistance in *P. aeruginosa* with meropenem (Tam et al. 2007b). AUC/MIC was the important index for preventing emergence of

derepressed AmpC-resistant mutants to ceftizoxime in an *Enterobacter cloacae* and *Bacteroides fragilis* abscess model; the required magnitude value was 1,000 (Stearne et al. 2007). For linezolid dosing with anthrax, the PK/PD index for suppressing resistant mutants was the peak/MIC ratio (Louie et al. 2008). There are no fractionation studies to identify which PK/PD index is of major importance for tetracyclines and macrolides.

Clinical data on the suppression or reduction in the emergence of resistance are primarily limited to the fluoroquinolones (Thomas et al. 1998). With 24-h AUC/MIC values less than 100, 12 of 14 patients (86 %) treated with ciprofloxacin monotherapy developed the emergence of resistant mutants. With 24-h AUC/MIC values greater than 100, only 4 of 36 patients receiving ciprofloxacin alone developed resistance. With combination therapy of the fluoroquinolone with a β-lactam antibiotic, even fewer patients (1 of 26 or 4 %) developed resistance.

Applications of PK/PD

PK/PD modeling has proved useful for many different applications. It has been very useful in establishing new optimal dosing regimens to treat serious infections in patients and to identify drug exposure measurement that can enhance efficacy and reduce toxicity. Pharmacodynamics has also been useful to the pharmaceutical industry to develop new antimicrobials or newer formulations. Organizations determining susceptibility breakpoints for clinical laboratories have incorporated pharmacodynamics into their decision procedures. Similarly, organizations establishing guidelines for empiric use of antimicrobials and hospital formulary committees commonly use pharmacodynamics to make their drug recommendations or selection.

New Optimal Dosage Regimens

Once-daily dosing of aminoglycosides has been compared with multiple-daily dosing in numerous clinical trials. Turnidge (2003) reviewed all the ten published meta-analyses which showed a small but significant improvement in outcome with more than half of the studies. A high peak concentration of 8–10 times the MIC was associated with reduced mortality in gram-negative bacillary bacteremia and rapid resolution of fever and leukocytosis in gram-negative bacillary pneumonia (Moore et al. 1987; Kashuba et al. 1999). The analysis by Turnidge also showed that in three of ten meta-analyses nephrotoxicity occurred later with once-daily dosing than with multiple-daily dosing. This has led to clinicians dosing aminoglycosides for only 5–7 days than for longer periods of time.

Continuous or prolonged infusion of β-lactam antibiotics has enhanced their activity by lengthening the duration of time that the serum concentration exceeds the MIC of the infecting pathogen. There are several examples of better outcome

with continuous infusion over intermittent dosing of certain β-lactam antibiotics (Brodey et al. 1979; Hughes et al. 2009; Lorente et al. 2009). However, systematic reviews have not demonstrated any enhanced outcome with continuous infusion over intermittent dosing (Roberts et al. 2009). On the other hand, a prolonged 4-h infusion of piperacillin–tazobactam has resulted in a better outcome in critically ill patients than observed with 30-min infusion of the same drug or other broad-spectrum cephalosporins and carbapenems (Lodise et al. 2007; Yost et al. 2011). While prolonged infusions of doripenem has exhibited greater activity against organisms with high MICs in neutropenic murine-infection models (Crandon et al. 2009; Bulik and Nicolau 2010), there are no clinical trials demonstrating better outcomes in patients with prolonged infusion of doripenem or other carbapenems. In Chap. 10, this is more fully explored.

In regard to adverse effects, pharmacodynamic analysis in patients receiving daptomycin observed that an increased probability of a rise in the creatine phosphokinase (CPK) due to myopathy was linked to a trough concentration higher than 24 mg/L (Bhavnani et al. 2010). This information along with efficacious AUC/MIC profiles were used to design optimal daptomycin dosing schedules for patients receiving thrice-weekly hemodialysis (Patel et al. 2011).

Development of New Antimicrobials and Formulations

The Food and Drug Administration (FDA) as well as the European Medicines Agency (EMA) encourage pharmaceutical companies to use pharmacodynamics for selecting dosage regimens for Phase II and III clinical trials of new antimicrobials or new formulations. For instance, the goal of the 14:1 amoxicillin–clavulanate formulation was to provide time above MIC values of 35–40 % for *S. pneumoniae* and *Haemophilus influenzae* strains with MICs of 4 and 8 mg/L. Its efficacy in eradicating these strains was confirmed in double typanocentesis studies in young children (Jacobs et al. 1999).

Susceptibility Breakpoint Determinations

The Subcommittee on Antimicrobial Susceptibility Testing of the Clinical and Laboratory Standards Institute (CLSI) and the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST) both use pharmacodynamics in their decision process for establishing susceptibility breakpoints. The increasing frequency of penicillin-intermediate and -resistant strains led to a re-evaluation of breakpoints for oral β-lactams. The new breakpoints were based primarily on the MIC that would provide at least 40 % time above MIC with standard dosing regimens (Gums 2003). A similar technique was used to increase the susceptibility breakpoint for *S. pneumoniae* in non-meningitis infections such as pneumonia or

peritonitis. As stated before, the time above MIC required for efficacy with cephalosporins against wild type Enterobacteriaceae and with strains producing ESBLs is usually around 50 % of the dosing regimen for both groups of organisms. Thus, the important determinant for deciding susceptibility is the time above MIC with standard doses of the drug, not whether the organism contains a resistant mechanism (Kahlmeter 2008). With this in mind, CLSI and EUCAST have lowered cephalosporin breakpoints using 50 % time above MIC based on the usual dosage regimens used in the USA and Europe. The new CLSI susceptibility breakpoints are 1 mg/L for cefotaxime and ceftriaxone, 4 mg/L for ceftazidime, but still 8 mg/L for cefepime.

Guidelines for Empiric Therapy

The ability of the magnitudes required for efficacy for different PK/PD indices to predict efficacy of treatment in human infections has allowed expert committees to use pharmacodynamics in guiding physicians for initial empiric therapy. Guidelines for bacterial sinusitis, community-acquired pneumonia, and hospital-acquired and ventilator-associated pneumonia all used pharmacodynamics in their recommendations (Rosenfeld et al. 2007; Mandell et al. 2007; ATS/IDSA Guideline Committee 2005). The Infectious Disease Society of America, the American Society of Health-System Pharmacists, and the Society of Infectious Disease Pharmacists recommend aggressive vancomycin dosing in serious staphylococcal infections (Rybak et al. 2009). They have recommended attaining 24-h AUC/MIC values of 400 or greater along with trough levels of 15–20 mg/L. In a recent retrospective analysis of 320 patients with methicillin-resistant *Staphylococcus aureus* bacteremia, clinical failure was significantly lower at these high AUC/MIC values, and the incidence of nephrotoxicity was not significantly higher at the recommended trough concentrations (Kuller et al. 2011).

Formulary Development

While antibiotic costs have a major impact on formulary development, pharmacodynamics has also been important for selecting antimicrobials to be added to the hospital formulary. One can use PK/PD-derived susceptibility breakpoints to determine which antimicrobials would have the best chance of treating various bacterial strains isolated over time at the hospital (Andes and Craig 2005b). There are also numerous Monte Carlo simulations, integrating the variation in pharmacokinetics in patients and the MIC from large organism surveys, to estimate the probability of attaining the PK/PD target for efficacy (Roberts et al. 2011; Eagye et al. 2009) in specific populations. Drugs with the highest target attainment against specific organisms would be added to the formulary.

Conclusions

Since the early appreciation of differences in the time course of antimicrobial activity, much has been learned about the pharmacodynamics of antimicrobials. Specific PK/PD indices have been identified which are of major importance for efficacy and for the prevention of the emergence of resistance. Of major importance, the magnitudes of these PK/PD indices for efficacy have been shown to be very similar in animal infection models and human infections. Modeling has also identified that there are few differences in the index magnitude with different dosing intervals, among drugs within the same antimicrobial class (providing free drug concentrations are used), with different infection sites (except occasionally for pneumonia), and among susceptible and resistant strains of the same type of bacteria. Addition studies have shown that the magnitude of indices can increase significantly with a higher inoculum for *S. aureus* and that neutrophils have a minor enhancing effect on antimicrobial activity against Enterobacteriaceae but a more variable enhancing effect on activity against *S. pneumonia* for different antimicrobials. Pharmacodynamic modeling has many applications including establishing new optimal dosing regimens, developing new antimicrobials and formulations, determining susceptibility breakpoints, providing guidelines for empiric therapy, and formulary development.

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Chapter 2
In Vitro and Animal PK/PD Models

William A. Craig

Abstract A large variety of in vitro and animal models have been used to characterize the pharmacodynamics of antimicrobials. In vitro kill curves report two different patterns of antimicrobial killing (concentration dependent and time dependent) that can be followed by persistent effects that delay bacterial regrowth. In vitro kinetic models using dilution or dialysis have the ability to simulate the changing drug concentrations observed in humans and study their effect on different bacteria. New hollow-fiber dialysis models have reduced the chance of contamination and have allowed longer studies of the emergence and suppression of resistant mutants. Animal models have the advantage of determining antimicrobial efficacy at specific body sites such as the thigh in mice, the peritoneum in mice and rats, the lung in mice, rats, and guinea pigs, endocarditis in rabbits and rats, and meningitis in rabbits. However, clearance of antimicrobials is more rapid in animals than in humans. Many factors, such as inoculum, media, growth-phase of the organism, site of infection, drug concentrations to measure correct drug exposure, presence of neutropenia, and measurement of outcome by colony-forming units (CFUs), survival/mortality, or another form of assessment, need to be considered to develop meaningful conclusions.

Keywords Animal models • Murine thigh-infection model • Animal peritonitis models • Animal pneumonia models • In vitro dilution models • In vitro dialysis models • Hollow fiber dialysis models

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Introduction

There are a large number of in vitro and animal models that have been used to characterize the pharmacodynamics of various antimicrobials. Many of the early in vitro studies were concerned with the kinetics of antimicrobial activity and the mechanism of action of the drug (Garrett and Miller 1965). Even most of the animal models were initially designed to document in vivo activity of an antibiotic rather than to determine the optimal way to dose the drug. Still in the early 1950s a few researchers, such as Harry Eagle, started using animal models to evaluate different dosing regimens to characterize the important pharmacodynamic characteristics of an antibiotic (Eagle et al. 1950). This chapter will review the major in vitro and animal models that have been used for pharmacodynamic assessment. It will outline the major factors that need to be considered to develop meaningful conclusions. These include inoculum, media, growth-phase of organism, site of infection, drug concentrations to measure correct drug exposure, immunologic status of the animal, and measurement of outcome by colony-forming units (CFUs), survival/mortality, or some other form of assessment.

In Vitro Models

In Vitro Time–Kill Curves at Increasing Concentrations

The first in vitro study performed to characterize the time course of bactericidal killing of different antimicrobials used killing curves at increasing drug concentrations. Even back in the 1940s, different patterns of antimicrobial killing were observed between antimicrobials such as streptomycin and penicillin with *Staphylococcus aureus* (Garrod 1948). Increasing the concentration of streptomycin 10- and 100-fold resulted in much faster killing at the higher concentrations. On the other hand, increasing the concentration of penicillin 10-, 100-, 1,000-, and 10,000-fold did not increase the rate of bactericidal activity at all. This led to the classification of drugs as those exhibiting concentration-dependent killing and those with concentration-independent killing (Shah et al. 1976; Vogelman and Craig 1986). Figure 2.1 illustrates the killing curves for different concentrations of tobramycin and ticarcillin against a standard strain of *Pseudomonas aeruginosa*. Increasing the concentration of tobramycin resulted in steeper slopes for the killing curve even up to a concentration that was 64 times the MIC. Increasing the concentration of ticarcillin from one-fourth to 4 times the MIC also increased the extent and the slope of the killing curve. However, at higher concentrations, the rate of killing as reflected by the slope was very similar. The only reason for slightly lower bacterial numbers at the higher concentrations is that killing started earlier as the concentration increased. With most beta-lactams such as ticarcillin there is a small range of concentrations that result in concentration-dependent

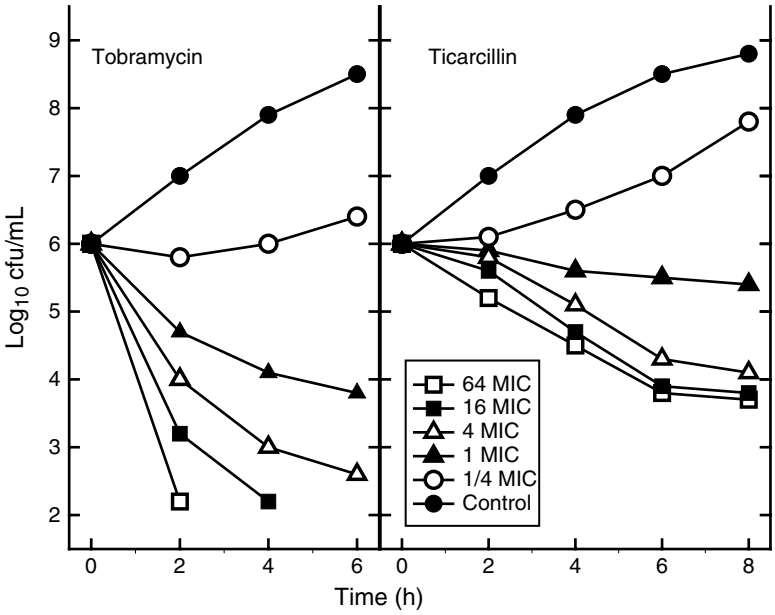

Fig. 2.1 Time–kill curves for *P. aeruginosa* ATCC 27853 with exposure to tobramycin and ticarcillin at concentrations from one-fourth to 64 times the MIC. Redrawn from Fig. 1 in Craig WA, Ebert SC. Killing and regrowth of bacteria in vitro: a review. Scand J Infect Dis 1991; Suppl 74:63–70

killing. However, once the concentration exceeds about four or five times the MIC, the rate of killing saturates and further killing at higher concentrations is largely concentration independent.

Persistent Effects

The standard method for measuring the in-vitro postantibiotic effect (PAE) is to expose the organism to the desired drug concentration for a few hours and then rapidly remove the drug by repeated washing, dilution, filtration, or drug inactivation (Craig and Gudmundsson 1996). Figure 2.2 illustrates a comparison of the PAEs following a 2-h exposure of *Staphylococcus aureus* ATCC 6538P in broth to 0.05 μg/ml of penicillin G using rapid drug removal by repeated washing, a 1,000-fold dilution, filtration, or the addition of penicillinase. The PAE values varied only from 1.4 to 1.6 h. The majority of investigators have used dilution as the method of drug removal. It is important to ensure that the extent of dilution is large enough so that any remaining drug fails to affect the growth of control organisms. Usually a 100-fold dilution is sufficient for concentrations near the MIC; 1,000-fold and 10,000-fold dilutions are required at higher concentrations. Repeated washing

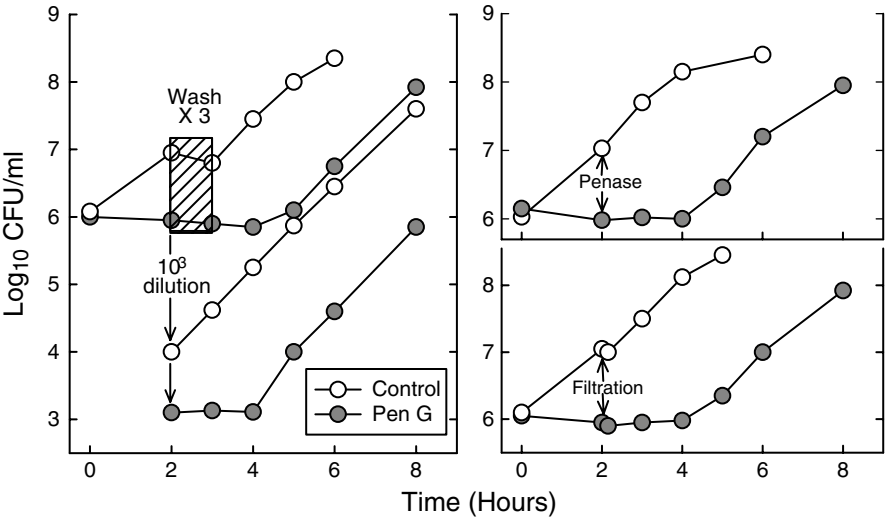

Fig. 2.2 Postantibiotic effects (PAEs) induced by a 2-h exposure of *S. aureus* ATCC 6538P in broth to 0.05 μg/ml of penicillin G using rapid drug removal by repeated washing, a 10^{-3} dilution, addition of penicillinase or filter filtration of the culture. Redrawn from Fig. 2.8.1 in Craig and Gudmundsson (1996)

procedures are dependent on whether one has a visible pellet after centrifugation. Simple decanting of the supernatant is done with a visible pellet, but removal of only about 90 % of the supernatant is recommended when no pellet is visible. Filtration requires a membrane filter with a pore size of 0.45 μm or less. The use of drug inactivation is most applicable to beta-lactams that are quickly destroyed by beta-lactamases.

Viable count measurement (colony-forming units/ml) is the primary method to follow microbial growth kinetics after drug removal. This methodology has been criticized because of the one-to-one assumption between a bacteria and a single colony-forming unit (CFU). For example, Gram-negative bacilli can be induced to produce filaments that contain more than 20 individual bacteria (Lorian et al. 1989). The filaments usually break up into multiple bacteria after drug removal. Optical density measurements usually required bacterial numbers greater than 10^6 CFU/ml. Some drugs have produced a good correlation between optical density measurement and viable counting. However, optical density underestimates the extent of killing by beta-lactams and aminoglycosides with Gram-negative bacilli resulting in longer PAEs than with viable counts (Bergan et al. 1980). Intracellular ATP content measured by bioluminescence not only has a sensitivity of 10^4 CFU/ml, but it also appears to give longer PAE values for bactericidal antibiotics (Hanberger et al. 1990; MacKenzie et al. 1994). This occurs because some dead but intact bacteria still contain measurable intracellular ATP.

The in vitro PAE is measured by the following equation:

$$PAE = T - C \tag{2.1}$$

where T is the time required for the bacterial numbers to increase 1 $\log_{10}$ (or 10-fold) above the bacterial number immediately after drug removal and C is the time required for the untreated control culture to increase 1 $\log_{10}$ immediately after completion of the same method for drug removal that was used on the test culture (see Fig. 2.2). Growth after the initial 1 $\log_{10}$ is similar for control and antibiotic-exposed cultures.

Odenholt, Holm, and Cars (1989) demonstrated that the postantibiotic effect of penicillin with *S. aureus* could be prolonged with re-exposure to sub-MIC concentrations. They observed that the in vitro PAE increased from 2.4 h to 6–7 h with re-exposure to penicillin at 0.2 times the MIC. This phenomenon has been called the postantibiotic sub-MIC effect (Odenholt-Tornqvist et al. 1992). The sub-MIC exposure concentrations used in most of these studies have been 0.1, 0.2, 0.3, and 0.4 times the MIC. In general, the in vitro postantibiotic sub-MIC effects have been longer than the in vitro PAEs. With in vitro kinetic models, Lowdin and coworker (Lowdin et al. 1998) combined PAE and the postantibiotic sub-MIC effect by measuring the time for 1 $\log_{10}$ regrowth after the drug concentration fell below the MIC in the model. They called this the post-MIC effect and observed that its duration got smaller with longer durations of exposure. They concluded that most of the persistent effects after antibiotic exposure were due to sub-MIC effects. Den Hollander and colleagues performed actual measurements of PAE induced by tobramycin in an in vitro kinetic model and observed that the PAE got progressively smaller as drug levels fell and virtually disappeared by 12 h of exposure (den Hollander et al. 1998).

Re-exposure of bacteria in the PAE phase to supra-MIC concentrations of the same antibiotic does not alter the rate of killing (Odenholt et al. 1989). However, if the PAE phase was induced by a drug that inhibits protein synthesis, such as erythromycin or an aminoglycoside, subsequent killing on exposure to a beta-lactam antibiotic can be significantly delayed (Craig and Gudmundsson 1996; Gerber and Craig 1981). On the other hand, exposure of organisms in the PAE phase to leukocytes usually enhances the rate of killing of staphylococci, streptococci, and *E. coli* by most antibacterials (Craig and Gudmundsson 1996; McDonald et al. 1981). This phenomenon has been called the postantibiotic leukocyte effect (PALE). Organisms are exposed to the antibiotic for 10–30 min, washed, and then incubated with 10^6 leukocytes per ml for 2 h. Unexposed control organisms are similarly incubated with leukocytes for 2 h. PALE is expressed as the difference in the $\log_{10}$ CFU/ml between the pretreated and control organisms.

In Vitro Kinetic Models

In vitro kinetic models using dilution to reduce drug concentrations started to appear in the late 1970s. One simple model described by Grasso and coworkers (Grasso et al. 1978) consisted of two flasks (see Fig. 2.3). One flask was the reservoir of

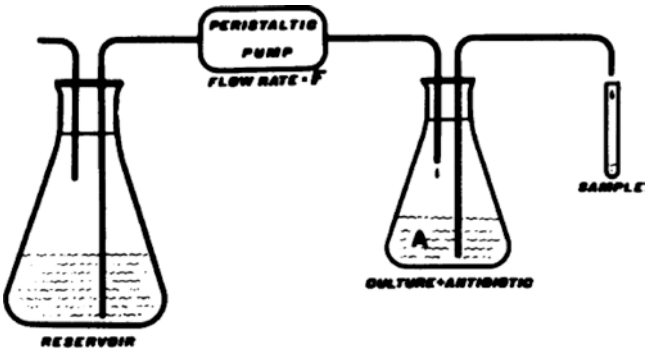

Fig. 2.3 Early dilution in vitro kinetic model. Republished with permission from Grasso et al. (1978)

broth to pump into the second flask which contained the antibiotic and the organism. They evaluated the activity of cephalosporins against *E. coli* and concluded that peak concentrations were not as important as the duration of exposure. Dilution models not only dilute drug concentrations, they also dilute the organism. This can be a problem for drugs with very rapid half-lives of 30–60 min, and the CFUs/ml measured should be corrected for the extend of dilution (Keil and Weidemann 1995).

Dialysis models using a permeable membrane or hollow fibers to separate two compartments started to appear in the early 1980s (Zinner et al. 1981; Toothaker et al. 1982; Ledergerber et al. 1985). Dialysis models were also designed to study the effects of drug combinations when the two antibiotics had different elimination half-lives (Blaser 1985). Initially these models were used to compare the efficacy of different dosage regimens. For example, the enhanced killing of once-daily netilmicin over thrice-daily dosing and continuous infusion of the same total about of drug was demonstrated in an in vitro kinetic model (Blaser et al. 1987; see Fig. 2.4). Emergence of resistant subpopulations was observed at lower doses of drug with thrice-daily dosing and continuous infusion, but not with once-daily dosing. Similarly, the improved bactericidal efficacy of continuous infusion of ceftazidime over intermittent dosing of the drug was also reported using an in vitro model (Mouton and den Hollander 1994). In vitro kinetic models are ideal for studying factors that support or prevent the emergence of resistance. The volume of the organism compartments in these models are many fold larger than in most animal infection models. Thus, the ability to detect small numbers of resistant bacteria is much greater with in vitro models than with animal models.

A variety of different broths have been used in these studies. Most of these provide a luxurious environment for bacterial growth. One needs to reduce the amount of broth to 5 % of the total fluid volume to observe the same bacterial growth rate as seen in animal models. However, bacterial killing in diluted broth is very similar to that in 100 % broth (Odenholt et al. 2007). Some investigators have added 5 % human albumin or 25 % human serum to simulate the effects of protein binding. For drugs with high protein binding, the addition of human albumin or serum reduces the

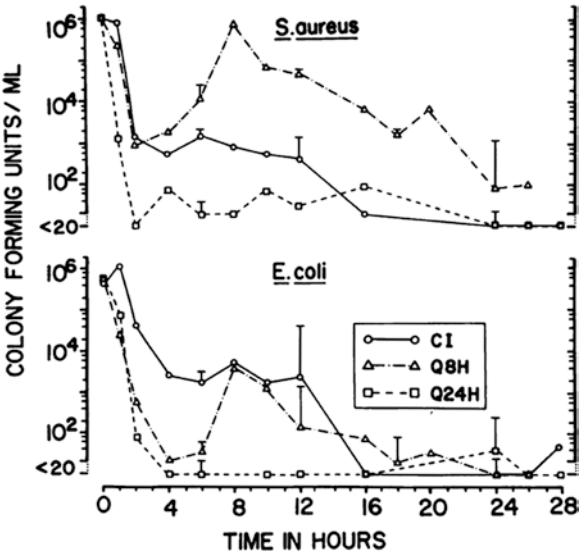

Fig. 2.4 Impact of once-daily dosing, 8-hourly dosing, and continuous infusion of the same total daily amount of netilmicin on the CFUs/ml of *S. aureus* in a diffusion in vitro kinetic model. Republished with permission from Blaser et al. (1987)

activity of the drug in these in vitro models (Odenholt et al. 2007; Garrison et al. 1990; Dudley et al. 1990). Thus, if one does not want to add albumin or serum, one should use the free drug concentrations observed in human volunteers or patients to simulate in the in vitro model.

The usual inoculum used in most of these studies has been 10^5–10^6 CFU/ml. For fluoroquinolones antibiotics studies have not shown much difference in activity even up to an inoculum of 10^9 CFU/ml (Firsov et al. 1999). However, beta-lactams have demonstrated a significant reduction in activity at very high inocula (Tam et al. 2009). To exhibit their bactericidal activity, these drugs need growing bacteria which are reduced in number at very high inocula. The activity of fluoroquinolones against *S. aureus* and *E. coli* observed in vitro kinetic models has also been similar when cultured under aerobic and anaerobic conditions (Wright et al. 2002; Noel et al. 2005).

Some of the early problems with these models were contamination of compartments with other organisms and sterilizing the apparatus for reuse (Reeves 1985). Despite the use of complex in vitro models that use multiple hollow fiber units simultaneously to compare different dosing regimens, contamination has become much less of a problem. Some studies have been continued for at least 15 days without contamination (Louie et al. 2012). This is very important for emergence of resistance in these models as maximal enrichment of mutants is dependent on the duration of simulated antibiotic exposure (Smimova et al. 2009).

A variety of different evaluation techniques have been used in these in vitro kinetic models. Simultaneous evaluation of multiple dosing regimens can identify the important PK/PD index for efficacy and for suppression of resistance. For linezolid against *Bacillus anthracis*, AUC/MIC was the major PK/PD index determining bactericidal efficacy, while C_{max}/MIC was more important in suppressing

resistance (Louie et al. 2012). Adding lower amounts of resistant organisms to susceptible strains in the same compartment can determine the value of a new dosing regimen in preventing the emergence of resistance (Knudsen et al. 2003). Studying multiple fluoroquinolones against a single organism can determine if the magnitude of the AUC/MIC to prevent emergence of resistance is similar with all drugs (Firsov et al. 2003). In general, most of the findings recorded with in vitro models have also been verified in animal infection models (Knudsen et al. 2003; Bonapace et al. 2002). This makes in vitro kinetic models a relatively reliable method for pharmacodynamic assessment of most antibacterials.

Specialized In Vitro Kinetic Models

The insertion of infected fibrin clots with a 10^9 bacterial density in an in vitro pharmacodynamic model was established in the mid-1990s to simulate treatment of endocarditis (Kang and Rybak 1995). Most of the studies have focused on treatment of *S. aureus* high inoculum infections, but some studies have also included penicillin-resistant *S. pneumoniae* and *Enterococcus faecalis* infections. The studies are usually conducted for 72 h with fibrin clots being removed a 0, 24, 48, and 72 h for determination of bacterial density.

The activity of antibacterials against intracellular pathogens was also developed in the mid-1990s (Hulten et al. 1996). A series of glass cell culture inserts containing 2-day grown monolayers of Hep-2 cells were connected to a pump with various tube diameters to simulate half-life of different drugs. The glass cultures are removed at different times, and the Hep-2 cells are washed and then lysed to measure intracellular activity. *Helicobacter pylori* was the initial organism studied and treatment with azithromycin and clarithromycin both resulted in significant bactericidal activity of the organism, while amoxicillin had no intracellular effect. The same model was used to evaluate to compare the activity of moxifloxacin and erythromycin against *Legionella pneumophila* (Tano et al. 2005). In this model moxifloxacin exhibited a significantly better antibacterial effect than erythromycin.

Animal Infection Models

There are clearly some differences between in vitro kinetic models and animal infection models. Animal models can look at infections in specific body sites. Animal models can also evaluate the effect of different host factors such as protein binding, complement, and leukocytes. However, major animal models for pharmacodynamic studies involve mice and rats which have much faster elimination of antibiotics than in humans. Intravenous catheters have been used (mostly in rats) for antibiotic administration to simulate human pharmacokinetics (Woodnut and Berry 1999). Multiple decreasing doses of drug have also been given subcutaneously to

mice to simulate a drug's serum profile in humans (Kim et al. 2008). For drugs with significant renal elimination, administration of uranyl nitrate at 5–10 mg/kg 3 days before treatment will cause a transient but stable renal impairment that can simulate the half-life of these drugs in humans (Andes and Craig 1998a; Nicolau et al. 2000).

Mouse Thigh-Infection Model

The mouse thigh as an infection model was initially used in 1952 by Selbie and Simon (1952) to measure the virulence of different strains of staphylococci. Mice rarely died of the infection with staphylococci and the measurement of thigh swelling in millimeters was used to assess the relative virulence of the different strains. Two years later, the model was used to evaluate antimicrobial efficacy and demonstrated similar success with different formulations of penicillin G (Selbie 1954). In 1960, the model was modified by placing two pathogens, a penicillinase- and non-penicillinase-producing *S. aureus*, into opposite thighs of the same mice (Acred et al. 1970). They were able to demonstrate effectiveness of methicillin and cloxacillin against both strains, while penicillin G was only effective against the non-penicillinase-producing organism.

Removal of the thigh with quantitation of bacterial numbers in thigh homogenates was started in 1973 with an in vivo evaluation of amoxicillin and ampicillin against *E. coli* and *Proteus mirabilis* (Hunter et al. 1973). Kunst and Mattie (1978) used the same thigh model with CFU determinations to study the relationship between in vitro and in vivo antimicrobial activity following short drug exposures. They observed some discrepancies between in vitro and in vivo antibacterial activity that could not be explained by differences in protein binding and drug kinetics. In 1982, Gerber et al. started to use neutropenic mice to provide more accurate assessment of drug–organism interactions and to allow for longer durations of study and the possible emergence of resistant mutants. One year later this model started to be used to evaluate the relative in vivo efficacy of different dosing regimens of antibacterials against specific pathogens (Gerber et al. 1983). Finally, in 1988, the same neutropenic murine thigh-infection model was used to correlate different pharmacokinetic indices (peak level, AUC, and time above MIC) with efficacy for various antibacterials against both gram-positive and gram-negative pathogens (Vogelman et al. 1988a). Over the subsequent 20 years, the neutropenic murine thigh-infection model has become the most standardized and accepted animal model for antimicrobial pharmacodynamic studies.

A variety of different mice, usually female and 6-week old, have been used for this model and all seem to give similar results when neutropenic mice are used. Neutropenia can be induced by irradiation or by cyclophosphamide (van't Wout et al. 1989). A commonly used regimen provides for two injections of cyclophosphamide at 150 mg/kg 4 days and 100 mg/kg 1 day before infection (Zuluaga et al. 2006). This regimen reduces the number of neutrophils to less than 10 mm^3 for at least 3 days. Many organisms will not grow well or actually die in normal non-neutropenic mice. For example, penicillin-resistant pneumococci will not grow in

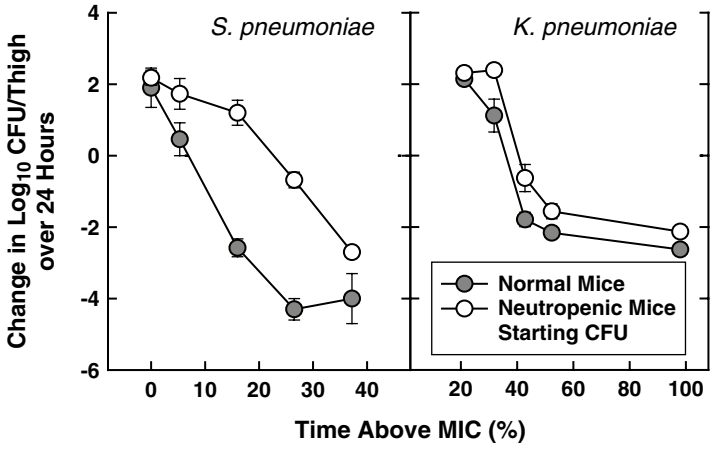

Fig. 2.5 Activity of ceftobiprole against *S. pneumoniae* ATCC 10813 (*left panel*) and *K. pneumoniae* ATCC 43816 (*right panel*) in the thighs of normal (non-neutropenic) and neutropenic mice. Republished with permission from Craig (2008)

normal ICR/Swiss or CD1 mice, but they do grow well in normal CBA/J mice (Tateda et al. 1996). It is recommended that an untreated organism grows at least 1.5 $\log_{10}$ CFU/thigh over 24 h when non-neutropenic mice are to be used. Sometimes higher initial inocula are required for adequate growth in normal mice (Drusano et al. 2010). Several organisms grow very well in both non-neutropenic and neutropenic mice. *S. pneumoniae* ATCC 10813 and *K. pneumoniae* ATCC 43816 are two such strains. Comparing the activity of an antibiotic against these strains in neutropenic and non-neutropenic mice allows one to measure the impact of neutrophils on activity. As shown in Fig. 2.5, neutrophils had a much greater effect on the activity of ceftobiprole against *S. pneumoniae* than *K. pneumoniae* (Craig and Andes 2008).

The starting inoculum can range from about 10^5 to 10^8 per thigh. This is produced by injection of slightly lower number of organisms in 0.2 ml into the thigh 2 h before treatment. Starting treatment earlier results in more rapid killing than seen if therapy is held until 2–4 h after infection. It also gives the organism time to grow so that at least 90 % of the organisms are in vivo grown before starting therapy. Several studies have shown that there is a minimal inoculum effect for most antibiotics against streptococci and Gram-negative bacilli as the starting inoculum is increased from 10^5 to 10^{7-8} (Andes and Craig 2005; Maglio et al. 2007; Lee et al. 2013). However, with staphylococci, most antibiotics show a 3- to 10-fold increase in the dose required for stasis as the inoculum increases from 10^5 to 10^7 (Lee et al. 2013). The highest increase was observed with vancomycin. Furthermore, the magnitude for PK/PD indices of efficacy in patients is similar to the values obtained in mice at the higher inoculum. The appearance and ultrastructure of staphylococci growing in vivo is similar to organisms growing on a surface or membrane (Lorian et al. 1985). This is much different than observed with in vitro models or in vitro kill curves at high inocula. Thus, staphylococci may show a major difference in the results for efficacy between in vitro kinetic models and animal models.

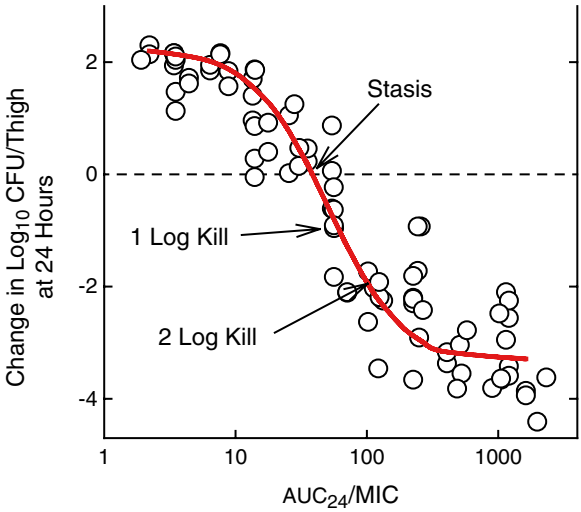

Fig. 2.6 Change in $\log_{10}$ CFUs/thigh over 24 h for various Enterobacteriaceae following treatment with multiple fluoroquinolones in neutropenic mice. Redrawn from data in Andes and Craig (2002)

Usually the CFU/g or thigh is correlated with the serum kinetics of the drug. Studies with microdialysis have demonstrated in rats and humans that the concentration of drug in muscle interstitial fluid is very similar to the free drug concentration in serum (Kover et al. 1997; Liu et al. 2002). Figure 2.6 shows the relationship between the change in the $\log_{10}$ CFU/thigh over 24 h for four fluoroquinolones against 2–4 different strains of Enterobacteriaceae and the 24-h area-under-the-curve (AUC) divided by the MIC. The data was examined by nonlinear regression using a sigmoid E_{max} model based on the four parameter Hill equation:

$$E = \frac{E_{max} \times AUC / MIC^{N}}{P_{50}{}^{N} + AUC / MIC^{N}}$$

where E is the observed effect (reduction in $\log_{10}$ CFU/thigh compared to 24-h controls, E_{max} is the maximum effect, AUC/MIC is the cumulative measure of drug exposure, P_{50} is a measure of potency indicated by the AUC/MIC producing 50 % of E_{max}, and N is a function describing the slope (Unadkat et al. 1986). A highly significant correlation of the change in $\log_{10}$ CFU/thigh with the AUC_{24}/MIC was obtained. The magnitude of the AUC_{24}/MIC for stasis, a 1 log kill, and a 2 log kill were 39 ± 4, 62 ± 7, and 105 ± 12, respectively.

The other major method of outcome analysis is using survival or mortality. In neutropenic mice with thigh infections, there is a very good similarity between the amount of daily drug required to protect 50 % of mice from death after 5 days of therapy and the total dose of drug to produce stasis after 24 h (Andes and Craig 2002). Figure 2.7 shows the mortality results for different dosing regimens of multiple fluoroquinolones against various Enterobacteriaceae and *P. aeruginosa* plotted against drug exposure measured by the AUC/MIC. There was 80–100 % mortality in untreated animals at the time of assessment. Furthermore, outcome was determined

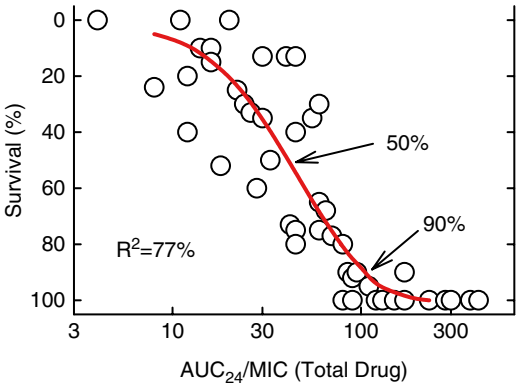

Fig. 2.7 Impact of AUC_{24}/MIC on survival of mice infected with Gram-negative bacilli after 2–7 days of therapy with various fluoroquinolones. Mortality in untreated mice was 80–100 %. Redrawn from data in Andes and Craig (2002)

within 24 h of the last dose of drug. Using nonlinear regression and the same sigmoid E_{max} model, the analysis shows that the AUC/MIC producing survival for 50 and 90 % of the animals was 41 ± 7 and 105 ± 16, respectively. These values are virtually identical to the AUC_{24}/MIC for stasis and 2 logs kill after only 24 h of therapy. This connection between CFUs/thigh and survival in animal infection models has strongly supported the application of these data to human infections.

The neutropenic mouse thigh-infection model has been used in dose fractionation studies with multiple drugs and organisms to identify which PK/PD index is the most important for antimicrobial efficacy (Vogelman et al. 1988a). Some drugs with long half-lives in mice need to compare 12-, 24-, 36-, and 72-h dosing for adequate dose fractionation (Andes and Craig 2007). This model has also been used to show that the in vivo postantibiotic effect is much longer than the in vitro PAE durations (Vogelman et al. 1988b). Furthermore, since mice have two thighs, normal growth of fresh organism reinjected into the opposite thigh during the in vivo PAE in the other thigh shows that all of the in vivo PAE is not due to sub-MIC concentrations. It is also seen on repeat injections of the antibiotic and at similar magnitude. Two thighs have additional advantages for comparing the same antibiotic exposure against two different organisms or for one organism at two different inocula (Lee et al. 2013).

Peritonitis Infection Model in Mice and Rats

Infection of the peritoneum by direct injection of bacteria was the earliest animal model used in antibiotic research and dates back to the early studies with Protosil. In 1949, Schmidt et al. used this model to infect Sprague–Dawley rats by using an inoculum of 10^4 CFU/ml of a virulent strain of *S. pneumoniae*. They then examined the role of the dosage regimen of penicillin G on animal survival after 4 days of therapy. The ED_{50} was similar for 2-, 4-, and 8-hourly dosing regimens, but increased progressively as the dosing interval rose to 12 and then to 24 h. Subsequent studies in both mice and rats have demonstrated marked variability in the inoculum required

in control animals to produce fatal infections. Sometimes 5 % mucin had to be combined with the inoculum to enhance infection. In many of these studies, therapy started immediately after infection and often consisted of only a single dose. Although different doses of antibiotics were associated with effective doses protecting 50 % of the mice from death (ED_{50}), there was little pharmacodynamic modeling in these mouse protection tests (Davis 1975; Acred et al. 1981).

In 1986, Frimodt-Moller et al. used another virulent strain of *S. pneumoniae* and standardized the inoculum at 10^6 CFU/ml (with 5 % mucin) to produce peritonitis in mice and compared the in vivo activity of 14 cephalosporins. The only correlation they observed was between the ED_{50} and the time serum concentrations exceeded the MIC. In additional studies in this model, the in vivo activity of the glycopeptides (vancomycin and teicoplanin) and linezolid as measured by the ED_{50} were best explained by the free drug C_{max}/MIC and free drug AUC/MIC, respectively (Knudsen et al. 2000; Sanberg et al. 2010). In the linezolid studies, peritoneal washouts were used to measure bacterial killing. Peritoneal cells were separated to examine intracellular killing. While linezolid had significant extracellular killing, its intracellular killing was very weak.

Drusano et al. (1993) used the same peritonitis model in neutropenic rats to evaluate the in vivo activity of a fluoroquinolone against *P. aeruginosa*. The rats were infected separately with the parent strain and with two resistant mutants and treated with the same total doses but fractionated for different dosing intervals. Overall efficacy against all the strains was most dependent on C_{max}/MIC and a ratio of 10:1 or higher given once daily gave the best results. Use of this model in rats has more recently been limited to comparison of the activity of different antimicrobials and immunologic responses than for pharmacodynamic studies.

Pneumonia Models in Mice, Rats, and Guinea Pigs

In the early days of antibiotics, pneumonia was primarily due to *S. pneumoniae*. Pneumonia models in mice were initially developed by intratracheal instillation of 100,000–250,000 pneumococci in 0.1 ml along with 5 % mucin (Schmidt and Walley 1951) or by intranasal instillation of around 0.05 ml of 10^8 pneumococci in lightly anesthetized animals (Azoulay-Dupuis et al. 1991a, b). Antibiotic therapy was started 18–24 h after infection and continued for 3–4 days. Outcome in these initial studies were measured by survival/mortality, but later CFUs/g or lung were recorded to define efficacy. Intrabronchial inoculation was much more common in rats to induce pneumonia (Bakker-Woudenberg 1979). Overall the efficacy in rats with various penicillins was similar to those obtained in mice (Woodnut and Berry 1999). Neutropenic mice or normal CBA/J mice were used in some studies to be able to determine accurate efficacy values for penicillin-resistant strains (Tateda et al. 1996; Scoriano 1996). Experimental pneumococcal pneumonia could also be induced by the aerosol route using an exposure chamber and a small particle nebulizer. Nuermberger et al. (2005) produced a low inoculum infection which did not have bacteremia when therapy was started. However, they needed neutropenic mice for growth of the low inoculum in control mice.

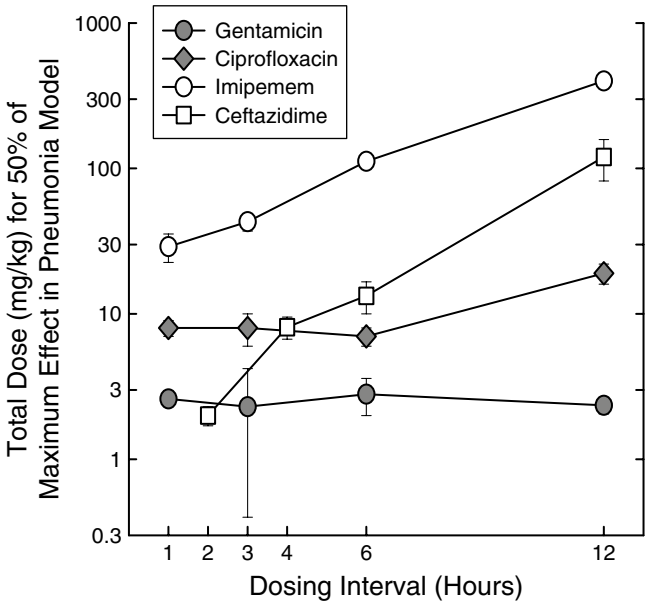

Fig. 2.8 Impact of the dosing interval for gentamicin, ciprofloxacin, imipenem, and ceftazidime on the 50 % effective dose (ED_{50}) against *K. pneumoniae* or *E. coli* in the lungs of neutropenic mice. Redrawn from data in Leggett et al. (1989, 1991)

Pneumonia with Gram-negative bacilli in neutropenic mice was initially produced by the aerosol route (Leggett et al. 1989). A Collison nebulizer generated the aerosol for 45 min in a closed container at a flow rate of 4–5 l/min. About 10^5 CFUs of *K. pneumoniae* were deposited in the lung from the original 10^9 inoculum. However, therapy was delayed for 14 h to get the starting inoculum up to 10^7. Bronchoalveolar lavage recovered 4–5 times more organisms than remained in the lung for the first 4 h, and rapid growth in the lung did not start until 8 h. Studies with various beta-lactams, aminoglycosides, and ciprofloxacin showed that the same PK/PD index that was important for each of the drugs in the neutropenic thigh model was also important in the neutropenic lung model (Leggett et al. 1989, 1991). This is illustrated in Fig. 2.8 where the static dose for imipenem and ceftazidime kept increasing as the dosing interval was increased from 1 to 12 h. This demonstrated that time above MIC was the important PK/PD index for these drugs in pneumonia. In contrast, the static dose remained unchanged for ciprofloxacin and gentamicin as the dosing interval was increased from 1 to 12 h, signifying that the AUC/MIC was the import PK/PD index. These studies also demonstrated that aminoglycosides and ceftazidime were more potent in the lung than the thigh. Imipenem showed equal efficacy in the two models, while cefazolin was less potent in the lung than the thigh. The efficacy of various antibiotics in normal mice required use of *K. pneumoniae* ATCC 43816, a strain that grows very well in non-neutropenic mice.

Gram-negative bacillary pneumonia in rats and guinea pigs was induced by intratracheal or intrabronchial administration of the inoculum (Pennington and Stone

1979; Roosendaal and Bakker-Woudenberg 1989). Outcome was initially measured by survival/mortality after several days of therapy. Antibiotic efficacy studies in guinea pigs were focused mostly on *P. aeruginosa*, where antibiotic therapy was started only 1 h after infection (Pennington and Stone 1979). Furthermore, the dosing regimen used resulted in variable drug exposure with very frequent dosing for the first 12 h followed by a single large dose for the second 12 h. Although differences in efficacy were observed with the various antibiotics, pharmacodynamic analysis is difficult because of the varied dosing regimens. *K. pneumoniae* ATCC 43816 is the major gram-negative bacillus studied in pneumonitis in rats. These studies have compared the efficacy of different antibiotics administered to neutropenic rats by continuous infusion or 6-hourly injections (Roosendaal and Bakker-Woudenberg 1989). The efficacy of gentamicin was similar with both dosing regimens, while ciprofloxacin appeared to be slightly more effective with intermittent dosing. On the other hand, ceftazidime was far more potent when administered by continuous infusion than by intermittent injections. However, the difference in the two methods of ceftazidime dosing were much smaller in normal, non-neutropenic rats than in neutropenic mice (Roosendaal et al. 1986). The same model showed that time above MIC was the major pharmacodynamic index for correlating with efficacy of ceftazidime over the first 48 h, but by 18 days the AUC/MIC was the more important PK/PD index (Bakker-Woudenberg et al. 2006).

Staphylococcal pneumonia model in BALB/c mice has been developed by oral instillation of 0.05 ml of a 10^9 suspension of organisms with 3 % gastric mucin (Crandon et al. 2010). Aspiration into the lungs occurred with the animal being held vertical for 30 s with the nares blocked. Antibiotic therapy was started 6 h later with starting inoculums of about 10^6 CFU/lung. Studies have documented the activity of vancomycin and telavancin against methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) strains with increasing MICs. The two drugs appeared active with all strain with MICs of $\leq$2 mg/l. However, there are no data at higher inocula which demonstrated a significant inoculum effect with staphylococci in the murine thigh-infection model. Other studies have correlated efficacy of tigecycline against various staphylococci with the free drug AUC/MIC (Koomanachal et al. 2009). The ratio of tigecycline concentrations in bronchoalveolar lavage (BAL) fluid to plasma also appeared to increase with increasing doses. The measurement of BAL fluid drug concentrations is increasing in all of the various animal pneumonia models in the hope that these concentrations can be pharmacodynamically linked to efficacy.

Other Animal Models

Endocarditis Models

Animal models of endocarditis are rarely used for pharmacodynamic modeling because infected vegetations are rarely sampled at the beginning of therapy and later values during therapy are compared with untreated control. A review of data in the literature from 19 models of experimental endocarditis in rabbits or rats infected

with various staphylococci, streptococci, and Gram-negative bacilli and treated with fluoroquinolones showed a significantly lower number of CFU/vegetation if the AUC/MIC was ≥100 (Andes and Craig 1998b). AUC/MIC was the primary PK/PD index-determining efficacy. Subsequent studies have evaluated the efficacy of once-daily combination therapy using human pharmacokinetics (Gavalda et al. 2002), evaluating the activity of new antimicrobials (Tsaganos et al. 2008), or determining the best antibiotic for specific resistant organisms (Boutoille 2009).

Meningitis Models

Experimental models of meningitis have been developed in rabbits, guinea pigs, and rats. However, virtually all of the pharmacodynamic studies have been performed in the rabbit meningitis model. Most experimental studies have focused on the rate of bactericidal killing in CSF. For example, maximal bactericidal rates of beta-lactams in rabbit meningitis required CSF concentrations that were 10- to 30-fold higher than the MIC (Tauber et al. 1984a). Other studies demonstrated that the duration of time CSF concentrations exceeded the MBC was the only index that independently correlated with the bacterial kill rate (Lutsar et al. 1997). To get maximum killing with ceftriaxone against *S. pneumoniae*, CSF concentrations needed to exceed the MBC for 95–100 % of the dosing interval. With ampicillin the time above MBC needed to be only about 40 % of the dosing interval to obtain sterile CSF (Tauber et al. 1984b). The investigators thought this was due to an in vivo postantibiotic effect with ampicillin against *S. pneumoniae*. However, this effect was due to active sub-MIC effects of the drug as injection of beta-lactamase into the CSF immediately resulted in regrowth of the bacteria.

The study of aminoglycosides in experimental meningitis is hampered by the poor penetration of these water-soluble drugs across the lipid blood–brain barrier. Still a comparison in experimental *E. coli* meningitis of the efficacy of increasing doses of gentamicin administered once or thrice daily for 3 days showed an excellent correlation with the cumulative AUC/MIC (Ahmed et al. 1997). Maximum bactericidal activity was observed at a cumulative AUC/MIC value of 50.

The evaluation of different dosing regimens of fluoroquinolone antibiotics has been limited primarily to experimental pneumococcal meningitis. In one study the PK/PD index for gatifloxacin with the highest coefficient of determination in correlation with efficacy was the AUC/MBC (Lutsar et al. 1998). Looking at results from multiple studies with different fluoroquinolones against *S. pneumoniae* in rabbit meningitis, maximal bacterial killing occurred at peak/MBC values of 10–30 and AUC/MBC ratios of 80–150 (Andes and Craig 1999).

Abscess Models

Stearne et al. (2001) developed an abscess model in Balb/C mice by injecting subcutaneously both *Bacteroides fragilis* and *E. coli* in 0.25 ml volumes into both flanks. Treatment with a fluoroquinolone (trovafloxacin) was started 3 days later

and continued for 2–5 days. The C_{max}/MIC ratio was the PK/PD index that best correlated with bacterial reduction for both organisms. A subsequent study used higher inocula of *B. fragilis* and *Enterobacter cloacae* that were similarly injected, but treatment was with multiple different dosing regimens of ceftizoxime (Stearne et al. 2007). Antibiotic therapy was started 30 min before injection of the two organisms (which would not allow for much initial in vivo growth before treatment) and continued for 24 h. They observed that the PK/PD index that best correlated with in vivo reduction of bacterial numbers of *E. cloacae* was the free drug AUC/MIC ratio. They also found that the same index correlated best with prevention of the emergence of resistant *E. cloacae* mutants to ceftizoxime. However, the magnitude of the index for prevention of resistance emergence was four times higher than for efficacy.

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Chapter 3
Setting Clinical MIC Breakpoints from a PK/PD Point of View: It Is the Dose That Matters

Johan W. Mouton

Abstract The approach to setting clinical breakpoints can be viewed from two angles: from the optimal dose point of view once a breakpoint is set or from a breakpoint view if the dose is already established. As to the first, in the development of new drugs two stages can be distinguished from a PK/PD point of view: the first stage involves finding an optimal dose based on preclinical studies, wild type distributions, and the pharmacokinetic behavior as determined from phase 1 studies. The second stage is aimed at confirming the validity of these dosing regimens in clinical trials. The alternative situation occurs when doses have already been set and used, breakpoints have been established in the past without the current pharmacodynamic understanding and tools that are available now, and clinical breakpoints need to be set (or harmonized, as has been the case with many drugs in Europe) from existing dosing regimens. Both approaches, and the interdependence between breakpoint and dose, are discussed.

Keywords Breakpoints • Pharmacodynamic target • Clinical trial

Introduction

Over the last decades, pharmacodynamics has started to play an increasing role in setting clinical breakpoints. Clinical breakpoints are used in clinical microbiology laboratories to categorize microorganisms as clinically susceptible (S), intermediate (I), or resistant (R). The laboratory report with the designations of S, I, or R for each antimicrobial agent provides guidance to clinicians to the potential use of antimicrobial agents in the treatment of patients. If a microorganism is categorized

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as susceptible to an antimicrobial agent, there is a reasonably good probability of success when the patient is treated with that antimicrobial agent, while failure of therapy is more likely when an isolate is categorized as resistant. The "intermediate" (I) category is used for various purposes, but mainly to indicate a degree of uncertainty in response or dose dependency (Kahlmeter et al. 2003, 2006; ISO 2006).

The approach to setting clinical breakpoints can be viewed from two angles. The first is during development of the drug. In that case, the exposure–response relationship is determined from preclinical studies, the wild-type (WT) distributions of the target microorganisms are considered, the pharmacokinetic profile in humans is determined and from these features the clinical breakpoint is set. In fact, to set the clinical breakpoint during the development of a drug, one could—or should—argue that the only factors that are relevant are the WT distribution (or other relevant MIC distribution) and the likelihood for emergence of resistance to the drug following certain criteria. It is the optimal dose that needs discussion and consideration that allows coverage of the WT up to and including the MIC breakpoint, rather than setting the breakpoint based on dosing regimens. In the development of new drugs two stages can then be distinguished from a PK/PD point of view: the first stage involves finding an optimal dose based on preclinical studies and the pharmacokinetic behavior as determined from phase 1 trials, but does not include phase 2 or dose finding studies in men. The second stage is aimed (or should be aimed; as this is not yet a fully established paradigm) at confirming the validity of these dosing regimens. The alternative situation occurs when doses have already been set and used, breakpoints have been established in the past without the current pharmacodynamic understanding and tools that are available now, and clinical breakpoints need to be set (or harmonized, as has been the case with many drugs in Europe) from existing dosing regimens. It is in those cases that pharmacodynamic approaches and evidence clearly shows that a number of existing clinical breakpoints were, or still are, overly high. In this chapter, these two approaches and examples are discussed.

Clinical Breakpoints for New Drugs: First Stage

Categorizing a microorganism as "susceptible" using a clinical S breakpoint implies—from the definition of Susceptible—that the dosing regimen that will be applied to patients covers the WT distribution of the target microorganisms. To define the optimal dosing regimen to cover the WT distribution, information needs to be available with respect to the PD and the pharmacokinetic profile of the drug, including measures of dispersion of that profile. A general approach of this procedure is shown in Table 3.1. In the procedure seven steps can be distinguished. In each step, information is collected that subsequently is integrated into the process. Ultimately this procedure will provide a good estimate of the dose and dosing regimen to be used in subsequent phase 2 and phase 3 clinical studies. It should be noted that the steps discussed below are iterative, and that whereas there is a certain sequence in the steps described, the results should be evaluated continuously during this process.

Table 3.1 General guideline for determining the dose of an antimicrobial agent to be used in phase 2 and/or 3 studies in humans. Step 4 and 5 may precede step 1

Step	Action	Methods
1	Establish PK/PD index that is correlated with effect of DRUG	Time-kill studies; preclinical studies in animals and IVPM; PD modeling
2	Establish the pharmacodynamic target of DRUG	Interpretation of models in step 1 (neutropenic vs. non-neutropenic animals; static effects; 1 or 2 log kill effects)
3	Determine protein binding in mice and in humans of DRUG	Protein binding in mice and men over full concentration range expected
4	Determine the wild type (WT) distribution of microorganisms to be covered	Epidemiological studies of target microorganisms (surveys)
5	Set the highest MIC that proposed dosing regimens are required to cover (usually the highest ECOFF of target microorganisms)	Review and interpret survey results
6	Establish the dose–exposure relationship of the drug	Phase 1 studies—single and multiple dose; dose escalation
7	Determine dosing regimens that cover target microorganisms	Population pharmacokinetic analysis; Monte Carlo simulations

Establish PK/PD Index That Is Correlated with Effect

The first step and one of the most important is to determine the PK/PD index that is correlated with antimicrobial efficacy. In general, this includes the use of animal models (Craig 1998, 2003), but in vitro pharmacodynamic models (IVPM) (Tam et al. 2006; MacGowan et al. 2011a, b) are increasingly used. The efficacy of an antimicrobial agent is dependent on the relationship between the MIC for the microorganism and the exposure of the microorganism to the agent in the patient (or other host). Clinical outcome is dependent on the triangular relationship between MIC, exposure, and efficacy (Mouton et al. 2011). In turn, exposure of the microorganism to the agent in the patient is dependent on the dose and the pharmacokinetic properties of the drug. For many agents the efficacy of the nonprotein-bound free (*f*) agent in serum is correlated with the area under the concentration–time curve (AUC) and inversely correlated with the MIC, or *f*AUC/MIC ratio. For other antimicrobial agents this relationship is different. For instance, for β-lactam agents it is not the *f*AUC/MIC relationship that best correlates with outcome but the time, expressed as a percentage of the dosing interval, that the concentration of the antimicrobial agent remains above the MIC for the microorganism, the % $fT>$MIC (see Muller Chap. 10; Craig Chap. 1). The underlying mechanisms that explain these relationships have been explored by several investigators and are primarily dependent on the relationship between growth rate and the dependency of the kill rate on antimicrobial concentrations (Mouton et al. 2007; Tam and Nikolaou 2011) (see also Muller Chap. 10; Craig Chaps. 1–2; Derendorf Chaps. 4–5).

Animal models most often involve neutropenic mice. Typically, these are infected with an inoculum of 10^6 colony-forming units (cfu)/mL of microorganisms in the thigh or lung. Treatment is then initiated and after 24 or 48 h the total bacterial count is determined for each organ. Using different doses and dosing intervals—so-called dose fractionation studies—ranges of exposure are obtained and are subsequently plotted against the number of cfu after treatment to establish exposure–response relationships. The plots and analysis there-of provide an indication which pharmacodynamic index—$\%T>$MIC, AUC/MIC, or C_{max}/MIC best correlates with outcome. In an IVPM, bacteria are exposed to simulated concentration-time profiles in an in vitro system. The advantages over animal models include examination of the effects of different half-lives (den Hollander et al. 1998) and studies at higher inocula (Tam et al. 2009). A more extensive discussion can be found in Craig Chap. 2; Derendorf Chaps. 4–5. The IVPM in particular is used to study the relationship between exposure and emergence of resistance.

Setting a Pharmacodynamic Target: Preclinical Studies

The relationship between a PK/PD index and response to treatment allows for the setting of a pharmacodynamic target (PDT). The PDT is the minimum value of the PK/PD index that is aimed for when treating patients and is based on both preclinical and, if possible, clinical drug/microorganism exposure–response relationships. The PDT ideally is the PK/PD index value that ensures a high probability of successful treatment. However, in drug development patient data are not yet available and these values are generally determined from exposure–response relationships in preclinical studies as described above. It was shown by Ambrose and colleagues that exposures required for microbiological and/or clinical cure in preclinical models and in humans were expectedly in a similar range (Ambrose et al. 2007), underscoring the use of preclinical models to estimate PDTs. Alternatively, if exposure–response relationships of antimicrobials from the same class are already established, these can be used for the new drug as supplementary evidence or even provide initial estimates of the PDT only to be confirmed by specific experiments. For instance, the PDT for various quinolones is virtually identical for each drug (see step 3) and it could be expected that a new quinolone would have the same pharmacodynamic properties as existing ones. Indeed, evidence that a new quinolone is different from existing ones would need significant experimental substantiation and subsequently clinical validation.

The relationship between exposure and response (cfu) can generally be described by a sigmoid curve such as the Emax model with variable slope (Fig. 3.1). There are several conclusions that can be derived from this relationship. It is obvious that increasing values of the PK/PD index result in an increased effect and that there is a value where a near maximum effect is achieved. The three key PK/PD index values that can be derived from this relationship are the PK/PD index value that results in a net static effect (no $\log_{10}$ drop in cfu) over 24 h of treatment (also called the in vivo

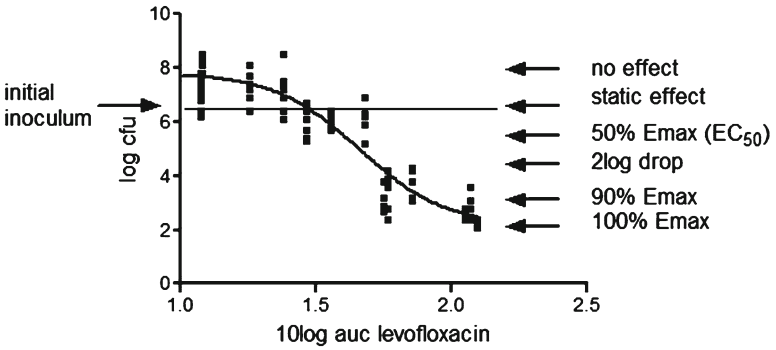

Fig. 3.1 Characteristic effect levels of a sigmoid dose response (*E*max) relationship (example for levofloxacin). The plot shows the relationship between *f*AUC and number of colony-forming units after 24 h of treatment in a mouse thigh model of infection. The static effect *line* indicates the *f*AUC required to result in no net change in cfu after 24 h of treatment. Two $\log_{10}$-drop indicates the *f*AUC required for a 100-fold reduction in cfu

static effect) and the values that results in a one $\log_{10}$ drop or a two $\log_{10}$ drop in cfu. There is at present no clear consensus whether to use the static effect or the one $\log_{10}$ drop or a two $\log_{10}$ drop as a target. Intuitively, the PK/PD index resulting in an in vivo static effect is the minimum value required when treating patients with an intact immune system, while a higher value may be required in patients that are immune deficient. Thus, the clinical indication of the drug is important here. If the slope of the sigmoid curve is relatively steep, the PDT values for static effect and log drops will be very similar.

Determine Protein Binding in Mice and in Humans

Although antimicrobial concentrations are usually determined as total concentrations, current evidence clearly indicates that it is only the unbound free fraction of an antimicrobial that is active. This was elegantly demonstrated by Craig (2003) by looking at the concordance in PK/PD index values required to reach a bacteriostatic effect for the free fraction of the drug and total drug. The effect of protein binding on the PK/PD index value is shown in Table 3.2 for several cephalosporins. The cephalosporins with very low protein binding, ceftazidime, cefpirome, and cefotaxime all display values of 38–40 % $T>$MIC, while if total concentrations of ceftriaxone, with a protein binding of 76 % in mice, is considered, values required are in the order of 70 %. If the unbound fraction of ceftriaxone is considered, the effect is consistent with the other cephalosporins in the table. Another example is the AUC/MIC ratio needed for static effect of the highly protein-bound quinolones gemifloxacin and garenoxacin. These are comparable to those of other quinolones only when the unbound fraction of the drug is taken into account, whereas much

Table 3.2 % over 24 h of *T*>MIC [mean (range)] required for a static effect after 24 h of therapy with four cephalosporins in a mouse model of infection for *Enterobacteriaceae* and *S. pneumoniae*

Drug	*Enterobacteriaceae* (%*T*>MIC)	*S. pneumoniae* (%*T*>MIC)
Cefotaxime	38 (36–40)	38 (36–40)
Ceftazidime	36 (27–42)	39 (35–42)
Cefpirome	35 (29–40)	37 (33–39)
Ceftriaxone total	38 (34–42)	39 (37–41)
Ceftriaxone unbound fraction	72 (66–79)	64 (69–78)

Modified from Craig (1995)

higher AUC/MIC ratios are required (Ambrose et al. 2003). A clear relationship between degree of protein binding and the 50 % effective dose of seven isoxazolyl antimicrobials in a mouse model of infection further substantiates this paradigm (Merrikin et al. 1983).

The effects of protein binding become an important issue when translating results in preclinical studies to probable outcome in humans. If the PK/PD target for a static effect is based on total concentration in, e.g., mice and protein binding is similar in mice and men, then the PK/PD target based on total concentrations is likely to be similar in the latter. An example is the PK/PD target for daptomycin that was established in mice. Protein binding in mice and men is high—in the order of 90 %—and virtually similar. The PK/PD target was therefore considered to be similar in men in ensuing discussions on breakpoints for daptomycin (EUCAST 2006). If the PK/PD target would have been set on free daptomycin, and the translation to dose in humans based on free drug, the conclusions would have been similar as those based on total drug. Of note however, in the early days of daptomycin, protein binding was not considered systematically in dose evaluations and this was suggested to be one of the major reasons the drug failed at the time as dosing regimens used clinically were based on total drug instead of free drug (Lee et al. 1991).

If there is a difference in protein binding between mice and men, the use of free drug concentrations becomes extremely important, because doses required for a certain PDT could be either over or underestimated. For example, protein binding of azithromycin and erythromycin is relatively low, <8 and 20 % in rodents, respectively (Shepard and Falkner 1990). However, azithromycin is around 50 % protein bound in human serum (Zhanel et al. 2001) and the effect of azithromycin would therefore be significantly overestimated in humans if total drug were considered. Unbound azithromycin concentrations were therefore also used to simulate concentration-time profiles in an IVPM to determine the PDT (Zhanel et al. 2003). It is not clear however, whether the relationship between degree of protein binding and efficacy holds for very high degrees of protein binding, that is above 98–99 %. At those very high values other factors than availability of free drug may start playing a significant role, such as the rate constants to receptors and the mass balance of free drug versus bound drug in general.

Determine the Wild Type Distribution of Microorganisms to Be Covered

The most important feature of the dosing regimen to be used is that the exposures will cover the target microorganisms. The target microorganisms are based on the clinical indication of the drug or, in some cases, only one target species. Because of this, it is necessary to establish the distribution of wild type (WT) of the target microorganisms as one very important principle in setting breakpoints is that clinical breakpoints should not divide WT MIC distributions (Kahlmeter et al. 2003, 2006). From the WT distribution of a species, the epidemiological cutoff value (ECOFF) is determined and this value represents the MIC value that definitely needs to be taken into account.

Set the Highest MIC That Proposed Dosing Regimens Are Required to Cover (Usually the Highest ECOFF of Target Microorganisms)

The wild type distributions of the species to be covered subsequently require consideration, and this includes the potential clinical indications of the drug. It may well be, that at this stage it is discovered that there are significant differences between species that were not readily observed initially. Importantly, all ECOFFs of all species that need to be covered for a certain indication need to be evaluated. The highest ECOFF of these may serve as an initial target MIC in subsequent evaluations.

Establish the Dose–Exposure Relationship of the Drug

In the earlier phase of development, before studies in men, the dose–exposure relationship is estimated from pharmacokinetic properties in other species such as rodents or dogs. Here from, an initial estimate can be made by extrapolation. However, the first dose–exposures will be determined in the dose escalation studies in phase 1 clinical trials. The results here-of provide several important attributes of the drug. These will not only provide indications whether the relationship between dose and exposure is linear or nonlinear and a description of this relationship but also give indications of the variability of drug exposure between patients. If pharmacokinetics is widely variable and/or nonlinear, then this is a clear indication that therapeutic drug monitoring (TDM) would be indicated in the clinical use of the drug, and this may or may not have an impact on the decision for further development. Unfortunately, this was not always realized in the past. For example, voriconazole has been used for years clinically, but the requirement of TDM for this drug is

only slowly becoming accepted (Bruggemann et al. 2008, 2011). Alternatively, if the variability is relatively small this provides a robust background for dose finding and/or dose validation studies. Together with the findings as discussed in the next paragraph, it may show that the exposure required for efficacy as concluded from PK/PD relationships would be too high with respect to adverse events resulting in the stopping of further development.

Determine Dosing Regimens That Cover Target Microorganisms: Expected Exposures and Monte Carlo Simulations

To determine the dose that adequately covers the wild type population of the microorganisms of interest in the target patient population, all the information collected in the previous six steps is used. The pharmacodynamic target from steps 1, 2, and 3 provide the means to calculate the effects of certain exposures in relation to the MICs of the target MIC as determined from the ECOFFs, and the dose–exposure relationship and protein binding in preclinical and clinical studies provide the exposures obtained after certain doses.

It should be taken into consideration that the probable expected exposure in a particular patient is not only dependent on dose and the pharmacokinetic parameters of the drug but also on the expected variation there-in. When a specific PK/PD index value is used as a PDT to predict the probability of successful treatment, this should be true for each individual patient within the population and not only for the population mean. Since the pharmacokinetic behavior differs for each individual, it is obvious that the PK part of the PI will differ concurrently and therefore the PI as a whole. There are therefore two iterative steps in this process: one is to determine the relationship between PDT and various dosing regimens, such as doses and the number of times a drug is given per day. The latter is particularly important for beta-lactam agents. The second is to take the variability in the population into account.

A simple method to get an initial estimate of the dose that covers ECOFF values is to plot the PK/PD index as a function of MIC for the dosing regimen of interest. For example, in Fig. 3.2 the $\%fT>\mathrm{MIC}$ for ceftobiprole is shown as a function of MIC for eight different dosing regimens (Mouton et al. 2004). By drawing a vertical line at the MIC on the *X* axis, the value of the PD index can be read from the *Y* axis from the intersection with the plot and subsequently one can determine which regimens are most promising. This method facilitates comparison of the effectiveness of different dosing regimens in attaining PDTs (Mouton and Punt 2001). This process is similar to the process of setting initial breakpoints, but in reverse (Mouton et al. 2012).

It was mentioned before that the design of the dosing regimen that should result in a certain PDT includes interindividual variability (intraindividual variability is usually smaller and is generally not considered or assumed to be included in interindividual variability). Since clinical data defining the full variability among

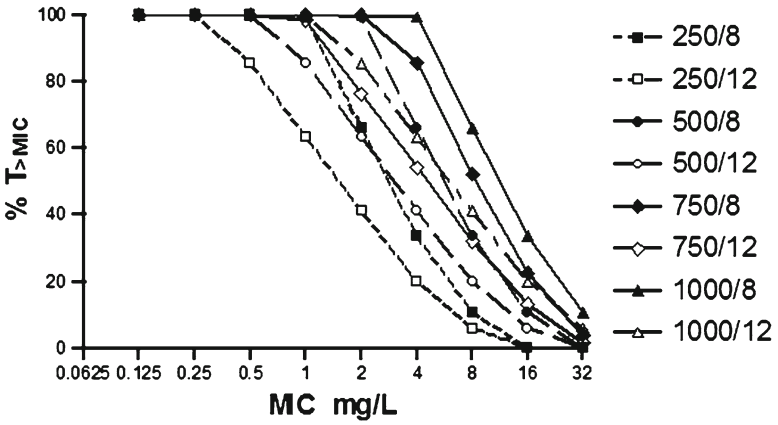

Fig. 3.2 Relationship between *%fT*>MIC and MIC for various dosing regimens of ceftobiprole. Illustrated is the close relationship between target MIC (usually the clinical breakpoint) and the dosing regimen. Adapted from Mouton et al. (2004)

individual patients is rarely available, a statistical approach is taken to simulate the to be expected variability within the target population as a whole .The statistical method most often used is Monte Carlo Simulations (MCS). This method was first used by Drusano et al. to present an integrated approach to population pharmacokinetics and microbiological susceptibility information at the FDA anti-infectives product advisory committee (Drusano et al. 2000, 2001). This method subsequently became a standard approach in the process of setting breakpoints and is being used by breakpoint committees (in particular the EUCAST and CLSI). There are several approaches to perform MCS (Bonate 2001). The most common method involves repeatedly drawing random parameter values for each of the pharmacokinetic parameter distributions, such as volume of distribution and clearance, based on its population mean and the standard deviation (SD), and construct simulated curves from those values following standard pharmacokinetic equations. Thus, the variability of pharmacokinetic parameters is used to simulate multiple concentration time curves. A typical simulation involves 5,000–10,000 subjects. As a control and a check that the random drawing of parameter values represents the original parameter distribution, the mean and SD of the simulations are compared with the original values and should be similar. The same applies to the generated curves, which can be compared with the original findings (Mouton et al. 2004). For each of the generated pharmacokinetic profiles, which are all slightly different because the input parameters vary to a degree in relation to the variance of the parameters, the value of the PK/PD index is determined for a range of MICs.

Two different methods are commonly used to display the results of MCS. The first approach is to plot or tabulate the probability of target attainment (PTA) of a PDT as a function of the MIC for a particular target or different targets. As an example, Table 3.3 displays the PTA for various PDTs for three different dosing regimens of ceftobiprole. Based on the PDT that is considered necessary and the MIC range

Table 3.3 Probability of target attainment (%) for various pharmacodynamic targets for three dosing regimens of ceftobiprole

	Dosing regimen											
	500 mg every 12 h				500 mg every 8 h				750 mg every 12 h			
Target MIC	*%fT*>MIC target											
(mg/L)	30	40	50	60	30	40	50	60	30	40	50	60
0.5												
1				100								100
2		100	100	72			100	100			100	99
4	100	59	1	0	100	100	99	79	100	100	78	15
8	0	0	0		80	13	0	0	69	3	0	0
16					0	0			0	0		
32												
Target 100 % (mg/L)	4	2	2	1	4	4	2	2	4	4	2	1

Adapted from Mouton et al. (2002, 2004)

that needs to be covered a dose can be selected that is most optimal. For instance, 100 % PTA is attained at an MIC of 4 mg/L with the dosing regimen of 500 mg t.i.d. using a PDT of 40 % *f%T*>MIC, but only at an MIC of 2 mg/L with the dosing regimen of 500 mg b.i.d. Depending on the target MIC of interest and the PTA required, either of the two regimens could be chosen to pursue in further studies.

As can be observed from Table 3.3, the PTA is close to 100 % at low MICs, and then decreases rapidly to 0 for higher MIC values. The acceptable level of PTA, however, is still under debate. Values of 99 %, 95 %, or 90 % have all been used. However, whereas 90 % is often used it implies that still 10 % of the population infected with a microorganism that has the MIC used to determine the pharmacodynamic index would probably not be covered optimally, that is, the PDT would not be attained. It then depends how much weight is given to the coverage of the MIC (see below).

An alternative approach to present the results of MCS graphically is presented in Fig. 3.3. In the figure, the value of the PI is plotted against the MIC with the 95 % percentiles as an approximation of the 95 % confidence intervals. This approach has the advantage that it graphically shows the total probability function irrespective of the target and therefore provides a more complete picture of the data (Mouton et al. 2004, 2005). MICs that can supposedly be covered with the dosing regimen can be read directly from the graph at the intersection of the horizontal line connecting the PDT and the lower confidence interval. If a CI of 80 % was chosen, this would correspond with the 90 % PTA of the method described above (the upper confidence limit not being important here). This method of displaying the data has the advantage that effect of choosing a different PDT can be observed directly and weighted against all the other evidence for setting a breakpoint and is most often used by the EUCAST (Mouton et al. 2012; EUCAST 2005). The PDT value that follows from the MIC to be covered then needs to be weighted against the precision of the PDT estimate, the width of the confidence interval, evidence from PK/PD studies, and the indication for use of the drug.

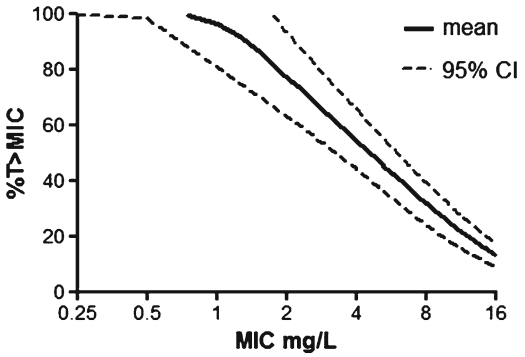

Fig. 3.3 The *%fT*>MIC of ceftobiprole displayed as a function of the MIC for a 750 mg dose b.i.d. The *solid line* represents the values for the mean of the population (cf. Fig. 3.2), whereas the *dashed lines* on both sides represent the confidence interval estimations (percentiles) of the mean values obtained by MCS. MICs that can be covered with the dosing regimen can be read directly from the graph at the intersection of a horizontal line connecting the PDT with the *dashed line* representing the lower confidence interval. Adapted from Mouton et al. (2004)

Clinical Breakpoints and Dosing Regimens for New Drugs: Second Stage

Traditionally, phase 2 studies include dose finding studies and phase 3 comparative trials to show at least non-inferiority of the new drug to existing agents. These are still used, but the emphasis has become more and more on validation of dosing regimens based on preclinical and phase 1 studies—the process as described in the first stage of the process earlier. The distinction between phase 2 and phase 3 therefore becomes more and more arbitrary. For instance, phase 2 studies are now sometimes divided in early phase 2 (phase 2a) just for dose validation only, and phase 2b is merged with phase 3. It should be noted that the process described here is from an efficacy point of view and that reasons for toxicity or recording adverse events may require another approach.

The results of phase 2 and phase 3 studies can subsequently be used to determine the exposure response relationship in clinical patients, provided that the data that are required for such an analysis are collected. Ideally, an estimate of exposure is available for every individual patient, as well as relevant outcome measures, such as microbiological or clinical cure. More refinement of outcome measures and including effect over time may increase the power of the study. For instance, Ambrose and colleagues collected serial cultures in patients with sinusitis and thereby were able to show exposure–response relationships in relatively few patients (Ambrose et al. 2008). In any case, the most important aspect here is that a PDI can be calculated for every single patient in the study as accurately as possible and thus involves both the pharmacokinetic part of the PDI as well as the MIC of the infectious microorganism(s). Because of the pharmacokinetic variation that invariably will occur between

patients, in particular in severely ill patients, sampling of blood for pharmacokinetic assessment will significantly improve the accuracy of the exposure estimate in the individual patient. For microbiological assessment, appropriate cultures should be taken and MICs determined from all (relevant) microorganisms. Having obtained all this information, an analysis of the exposure–response curve can performed using several methods. The first method is a specific analysis of the exposure–response relationship called Classification and Regression Tree Analysis (CART) and has often been used to analyze exposure–response relationships in clinical trials (Ambrose et al. 2001, 2003; Mouton et al. 2005; Bhat et al. 2007; Kashuba et al. 1999; Rodriguez-Tudela and Almirante 2007; Highet et al. 1999; Meagher et al. 2007; Bhavnani et al. 2006; Muller et al. 2013). This nonparametric method involves an algorithm of iterative splitting (recursive partitioning) and searches for the PK/PD index value that best discriminates between outcome categories, for instance failures and successes as outcome in clinical trials. The significance of classification can be tested using various statistical analyses such as the Fisher exact test. Since outcome is usually binomial (success or failure of treatment), a significant difference between classes indicates that the PK/PD index that defines the classes distinguishes between the class with a high probability of cure and the class with a low probability of cure, with cure being either microbiological or clinical cure. For example, in a study by Ambrose et al. (2003), an *f*AUC/MIC ratio of about 34 was found to distinguish between patients that responded well or poorly to fluoroquinolone treatment for pneumococcal infection and in the study of Muller and colleagues a $\%fT>$MIC value of 44.9 % was found for exposures to ceftazidime in patients with nosocomial pneumonia to distinguish (Muller et al. 2013).

The second method commonly used to identify the PDT examines the full exposure–response relationship and from this identifies a PDT. This requires either logistic regression or if the data allow it, the use of an Emax model (Rodriguez-Tudela and Almirante 2007; Muller et al. 2013). If a significant relationship exists, the PDI that results in at least 90 % cure or the maximum effect is then often taken as a PDT value and can subsequently be used to back calculate the MIC breakpoint value. However, either method suffers from the fact that studies are underpowered as many clinical trials do not have enough failures to perform such analyses, particularly for new agents. In clinical trials the dosing regimens chosen are based on obtaining a high drug exposure (based on the stage 1 process as described) while MICs usually are low, so few patients will have low exposures or infecting microorganisms with high MICs.

Clinical Breakpoints for "Old" Drugs

The setting of clinical breakpoints for older antimicrobials is not much different from that of new ones, except that in general there is far less information available and dosing regimens already established. The iterations in the process are therefore restricted. In the past, antimicrobial agents were developed more on a trial and error

basis and many were licensed only based on the presence of adverse events and/or toxicity (Podolsky 2010). Accordingly, for these drugs the information to optimize dosing regimens using exposure–response relationships is not readily available if at all, and it is not always clear whether the current dosing regimens used are optimal or even efficacious. Even if comparative trials were performed in the past to determine whether one antibiotic was non-inferior or superior to another, the dosing regimens have often been changed over time. These changes in dosing regimens pose a significant problem because older off-patent antibiotics are increasingly being prescribed to patients now that emerging resistance creates an increasing challenge in antimicrobial treatment of Gram-negative bacteria in particular. In these cases, breakpoints require reevaluation, or dosing regimens need reevaluation. The information that is available to perform such reevaluations varies widely by drug and drug class. Ambrose et al. (2007) in compiling data from preclinical and clinical studies showed that PK/PD index values derived from studies in animals and those obtained by CART analysis were very similar and for many older drugs PDT are derived from preclinical studies because of a lack of clinical information. Fortunately, it has become evident that the pharmacodynamic properties of antimicrobials within a class are very comparable (e.g., Table 3.2), allowing the use of a similar PDT across the class. In that respect, there is similarity with the development of a new drug within an established class as discussed above.

The EUCAST as well as the CLSI have recently reevaluated breakpoints based on current pharmacodynamic principles. The EUCAST has documented most of this in rationale documents that are available from the EUCAST website, http://www.eucast.org. The rationale documents for fluoroquinolones provide a good example not only how breakpoints are established but also how the recommendation of certain dosing regimens was approached. Pharmacodynamic information was not always directly available for all agents, but since it had been shown in preclinical studies that there is no significant difference between fluoroquinolones in general, a similar PDT (*f*AUC/MIC) was considered for each member of the class. Using MCS for established dosing regimens, breakpoints were subsequently set. However, it appeared that for several of the fluoroquinolones the initial suggested breakpoint for pneumococci would divide the wild-type distribution. The breakpoint of levofloxacin was therefore adjusted, but at the same time included the statement that a higher dose should be used.

Conclusion

The setting of clinical breakpoints is an iterative process that involves both the dose [or exposure] and the MIC distribution of the microorganisms to be covered, which in turn depends on the indication of the drug. It is therefore, in particular for new agents, important to define the indications and determine whether the most frequent causes of the infections are all covered. This includes of course the evaluation of the WT distributions relative to exposure. It also includes the patient groups where

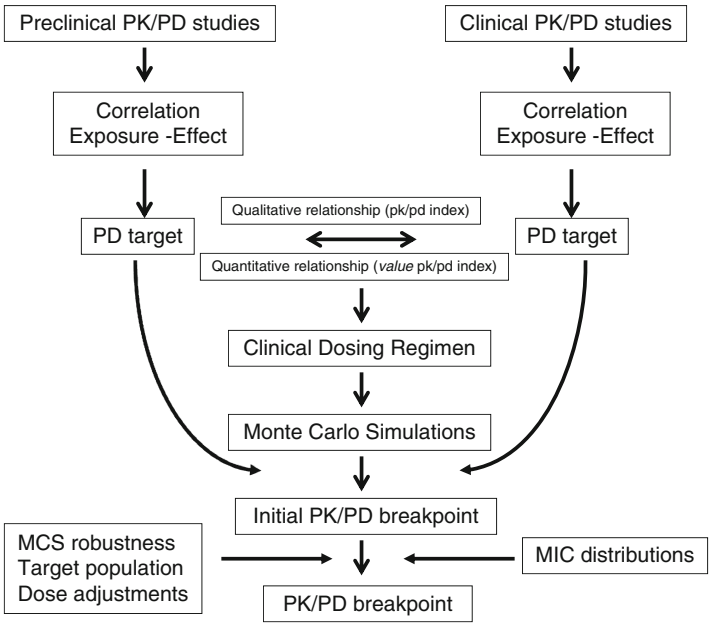

Fig. 3.4 Summary of the process of setting PK/PD breakpoints by EUCAST (Mouton et al. 2012)

the agent will be most used. For example, patients in intensive care units (ICU) generally have different pharmacokinetics with a higher volume of distribution and lower clearance than most other patients. The use of different pharmacokinetic parameters in the simulations will obviously result in different conclusions with respect to the breakpoints, as was shown in case studies for ceftazidime (Mouton et al. 2005) and for other agents (Roberts et al. 2009). MCS was performed using pharmacokinetic parameters from three different populations, human volunteers, patients with cystic fibrosis, and patients from the ICU. In each population the derived breakpoints would have been different. On the other hand, Muller et al. recently showed that the results of MCS based on volunteer data obtained from phase 1 studies matched actual target attainments in phase 3 studies (Muller et al. 2013) for ceftobiprole.

The entire process as described in this chapter can be summarized in a flow diagram as depicted in Fig. 3.4. The diagram represents the different elements as recently described by the EUCAST and includes both the steps as required for new agents as well as those for established drugs (Mouton et al. 2012). It should be borne in mind that breakpoints are not set in stone and that they are dependent on multiple factors. Should one of these factors change, then the breakpoint should be reconsidered and possibly be changed if necessary. The iterative process of optimizing dosing regimens and setting breakpoints continues after the breakpoint has been established.

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Chapter 4
Principles of Applied Pharmacokinetic–Pharmacodynamic Modeling

Benjamin Wu, Sherwin K.B. Sy, and Hartmut Derendorf

Abstract An effective dosing strategy for anti-infectives requires a thorough understanding of the complex interactions between drug, microbe, and the host immune system. Pharmacokinetic and pharmacodynamic (PKPD) modeling has been utilized to describe these relationships to aid the dose selection and dose optimization of antimicrobial agents. The complexity of PKPD models for anti-infective has increased over time with increasing improvement in in vitro methodologies, which have progressed from limited PD (a single minimum inhibition concentration measurement) to full PD analysis (dynamic kill curve). Capturing the time course of microbial dynamics in a kill-curve system provides an opportunity for complex PKPD modeling that has been used to evaluate challenging topics such as antimicrobial resistance.

Keywords Anti-infective • Anti-microbial • Pharmacodynamic indices • PKPD modeling and simulation • MIC • Kill curve • Time-dependent • Concentration-dependent

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PKPD Modeling in Anti-infective Research

PKPD modeling has been utilized in numerous therapeutic areas to predict and optimize dosing regimen, gain insight into explaining complex dynamics, test hypothesis, answer specific question, understand sources of variability, and make quantitative decisions. For anti-infectives, the PKPD relationship plays a crucial role in dose selection. The consequences of suboptimal dosing of antimicrobials can foster the emergence of resistant strains that can pose significant public health risks, including death. Recent trend has revealed that increasing ciprofloxacin usage leads to increasing incidences of ciprofloxacin resistance to *Pseudomonas aeruginosa*. The antimicrobial resistance has been observed for numerous drugs shortly after mass deployment, which warrants a closer examination of our current dosing strategies. In this chapter, the application of PKPD modeling in anti-infectives will be discussed.

MIC-Based Approach

Classification of antibiotics into time-dependent versus concentration-dependent killing has guided the dosing of antimicrobials for many years. This was achieved through relating drug exposure to minimum inhibition concentration (MIC). When the effectiveness of a microbial killing in vitro is dependent on time that the drug concentration is above the MIC, the drug is categorized into time-dependent killing (Craig et al. 1991; Drusano 1990; Turnidge 1998; Vogelman et al. 1988). When either the ratio of peak drug concentration to MIC (C_{max}/MIC) or total exposure to MIC (AUC/MIC) determines the effectiveness of the treatment, the drug is categorized into concentration-dependent killing (Table 4.1) (Craig et al. 1991; Leggett et al. 1989; Moise and Schentag 1998; Vogelman et al. 1988). Although this approach has guided dosing for various classes of antimicrobials and provided physicians a readily available tool for decision making, several shortfalls have been identified. Firstly, the complexity of drug and individual patient MIC relationship is often unknown since the laboratory screening of MIC, when determined, often occurs days following the initial treatment. It has been recognized that this oversimplified dosing scheme results in treatment failure and increases the likelihood of fostering the emergence of resistant populations.

Secondly, only the unbound drug concentrations are capable of exerting pharmacological effects. The PK profiles obtained from these analyses are usually total drug concentrations in plasma, ignoring the protein binding and tissue distribution information. Upon entering the blood stream, drugs can readily bind to proteins such as albumin, α-, β-, or γ-globulins, α1-acid glycoprotein, lipoproteins, and/or erythrocytes (Dasgupta 2007; Mouton et al. 2008; Treyaprasert et al. 2007). Protein binding can be either linear or nonlinear. Hence, characterization of protein binding across a range of drug concentrations can be informative. In particular for anti-infectives when the infected sites often occurs in the peripheral compartment

Table 4.1 Pattern of MIC-based PKPD index. Ambrose et al., Clin Inf Dis 44:79 (2007)

Antimicrobial agent	Bactericidal pattern of in vitro activity	PK–PD measure(s)
Aminoglycosides	Concentration dependent	AUC_{0-24}:MIC, C_{max}:MIC
β-lactams		
Penicillins	Time dependent	$T>$MIC
Cephalosporins	Time dependent	$T>$MIC
Carbapenems	Time dependent	$T>$MIC
Monobactams	Time dependent	$T>$MIC
Clindamycin	Time dependent	AUC_{0-24}:MIC
Glycopeptides/lipopeptides		
Daptomycin	Concentration dependent	AUC_{0-24}:MIC, C_{max}:MIC
Oritavancin	Concentration dependent	$T>$MIC, C_{max}:MIC
Vancomycin	Time dependent	AUC_{0-24}:MIC
Macrolides and clindamycin		
Azithromycin	Time dependent	AUC_{0-24}:MIC
Clarithromycin	Time dependent	AUC_{0-24}:MIC
Teilithromycin	Concentration dependent	AUC_{0-24}:MIC
Metronidazole	Concentration dependent	AUC_{0-24}:MIC, C_{max}:MIC
Oxazolidinones		
Linezolid	Time dependent	AUC_{0-24}:MIC
Quinolones	Concentration dependent	AUC_{0-24}:MIC, C_{max}:MIC
Tetracyclines		
Doxycyeline	Time dependent	AUC_{0-24}:MIC
Tigecycline	Time dependent	AUC_{0-24}:MIC

Note: AUC_{0-24}:MIC, the ratio of the area under the concentration–time curve at 24 h to the MIC; C_{max}:MIC, the ratio of the maximal drug concentration to the MIC; $T>$MIC, duration of time a drug concentration remains above the MIC

(i.e. skin infection), it is primarily the unbound fraction of drugs that crosses the membrane to the infected tissues such as the subcutaneous adipose tissues, skin, or skeletal muscles. An advanced methodology to overcome such problem is to utilize microdialysis as a technique to determine the free fraction of drug exposure at the site of infection. An example of implementing this methodology in the clinical setting is shown in Fig. 4.1, where the profiles of unbound ceftobiprole concentrations in different tissues are presented (Barbour et al. 2009b). Note that due to different unbound drug concentrations observed in plasma versus infected sites, an unoptimized dosing scheme could be proposed based on the total plasma drug profile alone, instead of the ideal scenario which is designed based on the free drug concentration.

Thirdly, the MIC-based PKPD modeling also rely on limited PD information. The single time point of MIC is empirical and assumes that it is stationary. The MIC value is laboratory dependent; dilution factors, laboratory condition, and technician's interpretation of what constitutes no growth can contribute to the inter-laboratory variability. The rate of bactericidal or bacteriostatic effect with changing drug concentrations is also unknown from such simplified approach. Multiple killing patterns can converge to the same MIC when only one time point is measured.

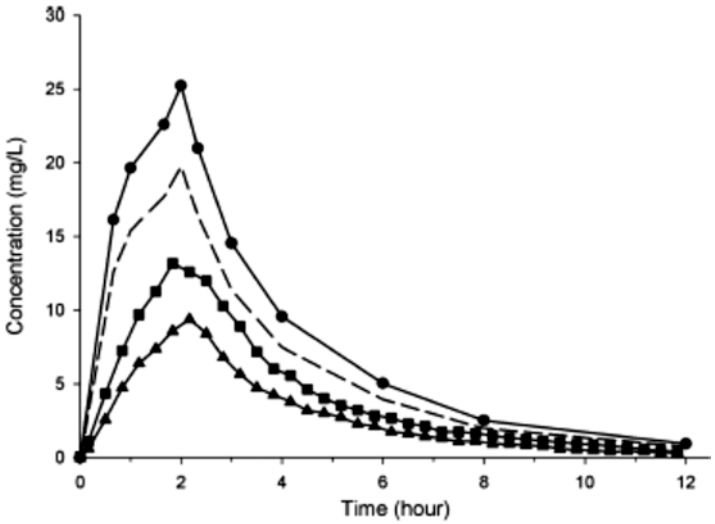

Fig. 4.1 Mean ceftobiprole concentration in plasma (*circles*), skeletal muscle (*squares*), and subcutaneous adipose tissue (*triangles*) over 12 h. The concentration of free drug in plasma (*dashed line*) was calculated based on the plasma protein binding of each individual patient. Barbour et al., AAC 53:2773 (2009)

With the increasing number of treatment failure for microbial infections, the MIC is likely changing with time. Thus, relying on a single snapshot of MIC to define the PKPD relationship for the entire treatment duration is misleading. Even without the dynamic kill-curve information, the inadequate MIC interpretations are sometimes revealed through post antibiotic effects or suboptimal effects (Hoffman and Stepensky 1999).

One additional pitfall of the MIC approach for PKPD modeling is that only static drug concentrations are used. Using only static drug concentrations does not accurately reflect the complex clinical observations. As a result, the PKPD relationship cannot be thoroughly understood by using only the empirical value of the MIC approach. In essence, dosing strategies that are based on total plasma drug concentrations and a single MIC value may contribute to the increasing number of treatment failure and emergence of antimicrobial resistance.

PKPD Modeling of Microbial Kill Curves

The complexity of PKPD models depends on the type of data generated from the experiments. Specifically, the kill-curve assay, where a time course of drug–bacterial response is produced, has been used in PKPD modeling to describe bacterial growth and death rates, drug effects, and the emergence of resistant strains within a population. In these in vitro kill-curve experiments, the antimicrobial concentrations can

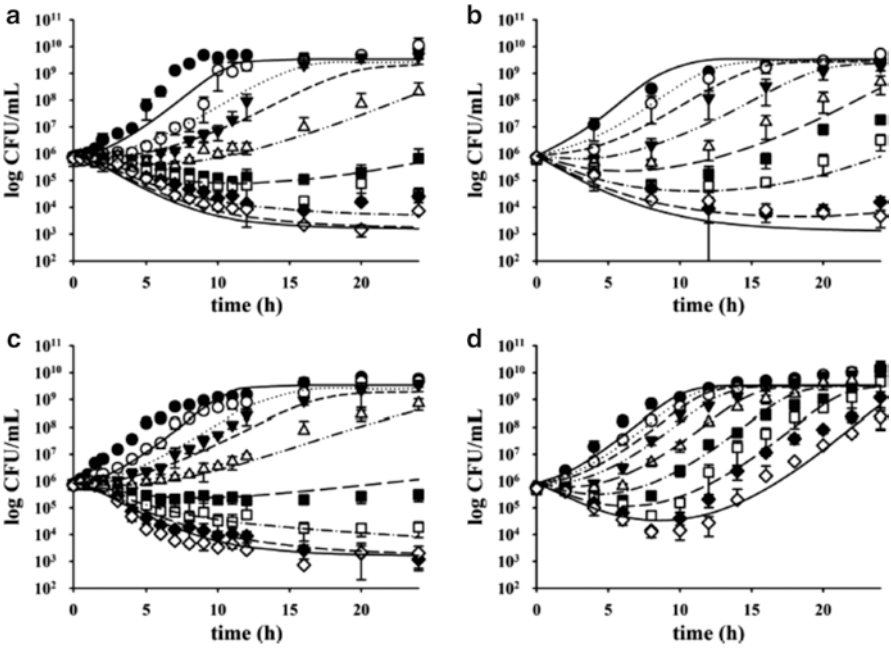

Fig. 4.2 Example of static kill curve. Schmidt et al., AAC 53:5039–5045 (2009)

be static (Garrett et al. 1966; Garrett and Nolte 1972; Mielck and Garrett 1969) or dynamic (Grasso et al. 1978; Sanfilippo and Morvillo 1968; Sanfilippo and Schioppacassi 1973), depending on the purpose of the study. The objectives of these experiments are to elucidate the time course of microbial response to controlled drug concentrations, whether it is static or dynamic. An example of kill-curve profile is shown in Fig. 4.2.

For ethical reasons, conducting clinical studies to evaluate PKPD relationships in anti-infective field has been limited. The dynamic kill-curve assay provides an alternative opportunity to evaluate PKPD relationships. The assay simulates the time course of clinically relevant unbound drug concentrations at the sites of action (PK) and measure the corresponding total bacterial cell counts (PD). The two main model frameworks which have been commonly used to describe microbial population dynamics include: (1) logistic growth model and (2) the compartmental model (also described as semi-mechanistic model).

The Logistic Growth Model

The PKPD models used to describe the in vitro bacterial population dynamics can be traced to models of human population dynamics. In 1838, Pierre-François

Verhulst described the logistic growth model that many of the modern anti-microbial PKPD models are based on

$$\frac{\mathrm{d}N}{\mathrm{d}t} = r\left(1-\frac{N}{K}\right)N \tag{4.1}$$

where r is the growth rate and K is the carrying capacity or the maximum number of individuals that is supported by the environment (Gershenfeld 1999). The solution to the Eq. (4.1) is derived by dividing both sides by K, such that

$$\frac{\mathrm{d}}{\mathrm{d}t}\frac{N}{K} = r\frac{N}{K}\left(1-\frac{N}{K}\right)$$

and then replacing N/K by x:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = rx\left(1-x\right) \tag{4.2}$$

The analytical solution to Eq. (4.2) is

$$N\left(t\right) = \frac{KN_0\mathrm{e}^{rt}}{K+N_0\left(\mathrm{e}^{rt}-1\right)} \tag{4.3}$$

The important property of this model is that the limit of this function as time goes to infinity is K, the carrying capacity: $\lim_{t\to\infty} N\left(t\right) = K$. In an in vitro kill-curve assay, limited nutrients and space capacity restrict the bacteria from growing indefinitely. The logistic growth model suitably describes this behavior.

The Compartmental Model

The second general type of antimicrobial PKPD model can be described in simplistic terms consisting of the natural self-replication and death of bacteria (Eq. 4.4):

$$\frac{\mathrm{d}N}{\mathrm{d}t} = \left(k_{growth}-k_{death}\right)N \tag{4.4}$$

where N is the bacterial population with the initial count of N_0, k_{growth} is the first-order rate constant for bacterial synthesis, and k_{death} is the first-order rate constant for bacterial death. This common structure to describe bacterial growth is also used in other disease area such as viral and tumor dynamic models, where a first-order self-replication rate is implemented. This model assumes that the bacteria are from a homogenous population with the same growth and death rate constants, which may not reflect the true population of microbes, which is known to select for resistant

strain in the presence of antimicrobial challenge. The variations based on the compartmental model have improved on this limitation and will be described more thoroughly in later section of this chapter.

Modifications on the Logistic Growth Model

To describe a capacity limited growth and also drug effect, Eq. (4.1) can be modified to include a drug effect function as follow:

$$\frac{\mathrm{d}N}{\mathrm{d}t} = k_{growth} N\left(1 - \frac{N}{N_{\max}}\right) - f_{death}\left(drug\right) \tag{4.5}$$

where the added f_{death}(drug) is a function to describe the effect of antimicrobial agent (Mouton and Vinks 2005; Mouton et al. 1997; Yano et al. 1998). In this equation, as N approaches $N_{\max}$, the growth term approaches a maximum over time and eventually reaches a steady-state condition, where there is no net change in the bacterial population. The drug effect is often represented by an $E_{\max}$ or sigmoidal $E_{\max}$ model such that

$$f_{death}\left(drug\right) = \frac{E_{\max} C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}} N \tag{4.6}$$

where C is the drug concentration at any specific time, $E_{\max}$ is the maximum drug effect, and EC_{50} is the concentration at which the half-maximum effect is achieved. The shape parameter, γ, is 1 in the $E_{\max}$ model and is a parameter in the sigmoidal $E_{\max}$ model.

During the initial growth phase where $N \ll N_{\max}$, Eq. (4.5) can be simplified to

$$\frac{dN}{dt} = \left[k_{growth} - \frac{E_{\max} C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}\right] N \tag{4.7}$$

By solving the analytical solution to Eq. (4.7), one can determine the number of bacteria at time (t) via

$$N\left(t\right) = N_0 \mathrm{e}^{\left(k_{growth} - \frac{E_{max} C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}\right) t} \tag{4.8}$$

Mouton and Vinks proposed that the stationary concentration, which is defined as the concentration at which the growth rate equals the kill rate and is also the point at which no net change in the number of bacteria is observed, can be derived from Eq. (4.8) (Mouton and Vinks 2005). By taking the natural log of the ratio $N(t)/N_0$ divided by time, which is equivalent to $k_{growth} - \frac{E_{\max} C^{\gamma}}{EC_{50}^{\gamma} + C^{\gamma}}$, one can obtain the equation for C:

$$C = EC_{50}\left[\frac{k_{growth} - \frac{1}{t}\ln\frac{N(t)}{N_0}}{E_{\max} - \left(k_{growth} - \frac{1}{t}\ln\frac{N(t)}{N_0}\right)}\right]^{\frac{1}{\gamma}} \tag{4.9}$$

When there is no net change in the number of bacteria, the term $\frac{1}{t}\ln\frac{N(t)}{N_0} \to 0$ and the stationary concentration (SC) is defined as

$$SC = EC_{50}\left[\frac{k_{growth}}{E_{\max} - k_{growth}}\right]^{\frac{1}{\gamma}} \tag{4.10}$$

The relationship between MIC and SC is described in greater details in Mouton and Vinks (2005).

A modification of the logistic growth model to study the separate effects of gentamicin and amikacin on the in vitro time–kill kinetics of *P. aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC BAA 747 (Tam et al. 2008) introduced an adaptation factor to the EC_{50} parameter by the following function:

$$f_{death}(drug) = \frac{E_{max}C^{\gamma}}{(\alpha\, EC_{50})^{\gamma} + C^{\gamma}}N \tag{4.11}$$

α is defined as

$$\alpha = 1 + \beta\left(1 - e^{-C\tau t}\right) \tag{4.12}$$

where τ is the exponent of the adaptation factor and β is the maximal adaptation. The function, $1 - e^{-C\tau t}$, behaves similar to a cumulative density function of an exponential family of distributions with a range of values between 0 and 1. The adaptation function α starts from a baseline EC_{50} and increase over time to a maximal value of β, if τ is positive.

Delay functions to both the growth rate and the drug effect function were introduced to the logistic growth model to describe the population dynamics of *Streptococcus pneumonia*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in the presence of azithromycin (Treyaprasert et al. 2007). The delay function acts as a modulator to allow the curves to conform to the S-shaped pattern of bacterial growth often observed during the first couple of hours of the time-kill kinetics in the presence of low concentration of antimicrobial agents. The delay function has the following form:

$$\frac{dN}{dt} = \left[k_{growth}\left(1 - \frac{N}{N_{max}}\right)\left(1 - e^{-xt}\right) - \left(\frac{k_{max}C}{EC_{50} + C}\right)\left(1 - e^{-yt}\right)\right]N \tag{4.13}$$

The function $1-e^{-xt}$ (Mouton et al. 1997) behaves like a cumulative density function starting from 0 at $t=0$ to a maximum value of 1 as $t\to\infty$. This function not only modulates the curve during the first few hours of the time kill curve but also shapes the transition to plateau after a significant decrease then increase at the antimicrobial concentrations that allow for bacterial regrowth to occur. A following study introduced a second compartment called the persistent bacterial population to differentiate from the first compartment of susceptible bacteria to model the effect of oxazolidinone on *Staphylococcus aureus* (Schmidt et al. 2009). Regardless of the number of compartments in the model, the shape of the time-kill kinetics is primarily dictated by the first compartment, the susceptible population, since the transformation rate constants are often very small.

A slightly more complex model based on the logistic growth model links the bacterial population dynamic to cell wall synthesis and drug effect of ceftazidime on cell wall synthesis to describe the lag time in bactericidal effect (Bulitta et al. 2009). Their study examines the inoculum effect of ceftazidime against *P. aeruginosa*. The model incorporates a natural first-order death rate dependent on the number of existing colony-forming unit (CFU) in the system and the logistic growth part of the model is dependent on both the cell wall synthesis and CFU. Given that the primary mode of action of ceftazidime is to inhibit cell wall synthesis, the drug effect acts on the compartment pertaining to cell wall synthesis:

$$\frac{dCW}{\mathrm{d}t}=\left[\left(1-\frac{C_B}{\mathrm{IC}_{50,VW}+C_B}\right)-CW\right]\cdot k_{out,CW}, \tag{4.14}$$

$$\frac{dN}{dt}=\left[k_{growth}\left(1-\frac{N}{N_{max}}\right)CW-k_{death}\right]N \tag{4.15}$$

where CW represents a hypothetical cell wall measurement, whose synthesis is expressed as a fraction of the baseline value. The $\mathrm{IC}_{50,\mathrm{CW}}$ is the concentration of ceftazidime in the broth that inhibits 50 % of cell wall synthesis and $k_{\mathrm{out,CW}}$ is the first-order rate constant for the cell wall turnover. The investigators claimed that this model accounts for the slow onset due to the delay between ceftazidime binding to the penicillin binding proteins and the depletion of cell wall components.

Examples of the Compartmental Model

The phase prior to reaching the plateau wherein the net bacterial growth is zero can be described by simple growth and death rates that are first-order rates dependent on the concentration of the bacteria present. Several strategies had been utilized to account for the decrease in the net growth rate as the system approaches this slower growth phase. One approach is to implement a phenotypic switch between susceptible and persistent population such that the resistant population has a reduced

growth rate (Balaban et al. 2004). The overall change in the total number of bacteria (A_{total}) would be the sum of the bacteria that are in susceptible (S) stage and those that are persistent or insusceptible resting state (R), such that

$$A_{total} = S + R$$

The transition between the two states is defined by their respective rate constants. An example of compartmental model is a two-compartment model to describe the in vitro effect of a number of antibiotics, including moxifloxcin, vancomycin, benzylpenicillin, cefuroxime, and erythromycin against *Streptococcus pyrogenes* (Nielsen et al. 2007). The investigators model the delay in effect of drugs using an effect compartment model for the drug. The drug effect is to stimulate the death rate of the susceptible state, assuming that the antimicrobials have no effect on the persistent population. The differential equations for the bacterial population are shown in Eqs. (4.16) and (4.17).

$$\frac{dS}{dt} = k_{growth}S - k_{death}S - k_{SR}S + k_{RS}R \tag{4.16}$$

$$\frac{dR}{dt} = k_{SR}S - k_{RS}R - k_{death}R \tag{4.17}$$

The transfer rate for the return to the susceptible state was assumed to be negligible and k_{RS} was fixed to 0. The transfer rate constant k_{SR} incorporates the growth-limited capacity using the following equation:

$$k_{SR} = \frac{k_{growth} - k_{death}}{B_{max}}(S + R) \tag{4.18}$$

where $B_{\max}$ is the maximum number of bacteria supported by the system.

The drug effect was evaluated in three different scenarios:

$$\frac{dS}{dt} = k_{growth}\left(1 - f\left(drug\right)\right)S - k_{death}S - k_{SR}S + k_{RS}R \tag{4.19}$$

$$\frac{\mathrm{d}S}{\mathrm{d}t} = k_{growth}S - \left(k_{death} + f\left(drug\right)\right)S - k_{SR}S + k_{RS}R \tag{4.20}$$

$$\frac{\mathrm{d}S}{\mathrm{d}t} = k_{growth}S - k_{death}\left(1 + f\left(drug\right)\right)S - k_{SR}S + k_{RS}R \tag{4.21}$$

where f(drug) is a sigmoidal $E_{\max}$ model to account for the effect of various drug concentrations.

In a follow-up study, the same group presented a mechanism for adaptive resistance in *E. coli* due to gentamicin by introducing two additional compartments that regulate resistance (Mohamed et al. 2012):

$$\frac{dAR_{off}}{dt} = k_{off} AR_{on} - k_{on} AR_{off} C \tag{4.22}$$

$$\frac{dAR_{on}}{dt} = k_{on} AR_{off} C - k_{off} AR_{on} \tag{4.23}$$

where AR_{off} represents the adaptive resistance in dormant stage and AR_{on} is for the active state; the transfer between states is represented by k_{off} and k_{on}; and C refers to gentamicin concentration. The additional two compartments provided greater flexibility for the model to adapt to the data trend. The investigators also noted that the model is suitable for gentamicin in the context of compartmental models.

Another compartmental model incorporated mechanisms involved in the life cycle of bacterial replication, autolysis, and drug effect (Bulitta et al. 2009). The model utilizes a mixture of the logistic and compartment type equations and variation of the turnover model to describe the time-kill behavior in the presence of antimicrobial agents. The life-cycle growth model involving two-stage susceptibility was applied to the study of linezolid against vancomycin-resistant *enterococci* and *S. aureus* (Tsuji et al. 2012a, b). A similar approach involving 3-state susceptibility was used to study colistin effect in *P. aeruginosa* (Bulitta et al. 2010).

Drug Effects

Antimicrobial drug actions are divided into two major categories, drug concentration-dependent or time-dependent killing. The concentration-dependent killing antimicrobials include aminoglycosides, quinolones, and metronidazole. Antimicrobials that exhibit time-dependent killing are mostly beta-lactams, macrolides, oxazolidinones, and tetracyclines. As described previously (Czock and Keller 2007), a minimal change in the rate of killing is often observed as drug concentration increase for time-dependent killing drugs. In contrast, the microbial kill rate increases substantially with increasing drug concentrations for the concentration-dependent killing. A linear drug effect on microbial population can be modeled by stimulating the killing ($1+f_uC_p$ on $k_{death}N$) or inhibiting the replication of bacteria ($1-f_uC_p$ on $k_{growth}N$), where f_uC_p is the free unbound drug concentration. An example of a drug stimulating the killing of microbes is shown in Eq. (4.24):

$$N = k_{growth} \times N - k_{death} \times N \times \left(1 + f_u C_p\right) \tag{4.24}$$

However, as is often the case, the drug effect is a nonlinear, saturable process. An example of direct drug effect on bacterial killing can be described by replacing the k_{death} with a modified Michaelis–Menten term $I_{max}f_uC_p/(IC_{50}+f_uC_p)$, where I_{max} is the maximum drug effect and IC_{50} is the concentration at which half of the maximal drug effect is observed. As described previously (Mueller et al. 2004), MIC value is related to this function by first integrating the equation:

$$\int_{N(0)}^{N(t)} \frac{1}{N} \times dN = \int_{0}^{t} \left(k_{growth} - \frac{I_{max} \times f_u C_p}{IC_{50} + f_u C_p} \right) \times dt \tag{4.25}$$

where t is the experimental incubation time for measuring MIC, $N(t)$ is the number of bacteria count at time t, and $N(0)$ is the initial inoculation count. Solving this equation, the MIC relationship can then be described as

$$MIC = \left(\frac{d}{I_{max} - d} \right) IC_{50} \tag{4.26}$$

where d is the equation, $k_{growth} - [\log(N(t)) - \log(N(0))]/t$. Although not as often done, the free drug fraction in these functions can be replaced with dose or AUC, yielding Eq. (4.27):

$$Effect = \frac{E_{max} D}{ED_{50} - D} or \frac{E_{max} \times AUC}{ED_{50} - AUC} \tag{4.27}$$

This method is generally less informative than integrating concentration-time course, because it reflects only the single parameter used. If modeling of both the k_{growth} and k_{death} is the goal, drug effects targeting k_{growth} or k_{death} can also be described using Eqs. (4.28) and (4.29):

$$\frac{dN}{dt} = k_{growth} \times \left(1 - \frac{I_{max} \times f_u C_p}{IC_{50} + f_u C_p} \right) \times N - k_{death} \times N \tag{4.28}$$

$$\frac{\mathrm{d}N}{\mathrm{d}t} = k_{growth} \times N - k_{death} \times \left(1 + \frac{S_{max} \times f_u C_p}{SC_{50} + f_u C_p} \right) \times N \tag{4.29}$$

where I_{max} and S_{max} represent maximum inhibitory or stimulatory rate and IC_{50} and SC_{50} represent the concentration of drug at half of the maximal effect. The diagram of these drug effects are shown in Fig. 4.3.

In the saturable drug effects, the fitting of pharmacodynamic curves may be improved by incorporating an exponential term, also known as the Hill factor (γ) to f_uC_p and either IC_{50} or SC_{50} of the drug effect term. The addition of Hill factor is for the purpose of improving the fit of the curve, because the Hill factor, by itself, has no biological meaning.

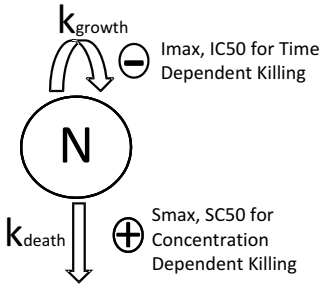

Fig. 4.3 Basic structure of PKPD compartmental model for antimicrobial agent inhibiting the growth (k_{growth}) or death (k_{death}) rate constants of microorganisms

The in vitro approach is limited by the lack of immune system and different nutrients available for bacterial growth. To overcome this limitation, the mouse thigh infection model has been used with the in vitro dynamic kill curve to model the PKPD relationship (Craig 1998; Jumbe et al. 2003).

Application and Limitation of Antimicrobial PKPD Models

When these PKPD models are used to describe kill curves of different drugs against specific bacteria, a quantitative comparison and prediction of drug effects can be evaluated. This is often first done using a static kill-curve model, in which the potency of the drugs (IC_{50} or SC_{50}), as well as the overall effects of the drugs (I_{max} or S_{max}), can be compared for the test article versus a control drug with equivalent MIC-fold exposure. For example, to compare a new antibiotic to gentamicin for the treatment of *P. aeruginosa* infection, the initial free drug concentrations at 0.125, 0.25, 0.5, 1, 2, 4, 8, 16× MIC can be compared. The drug effects can be quantitatively compared through the PKPD modeling approach.

Likewise, the effectiveness of an antimicrobial agent against different species of Gram-negative or Gram-positive microorganisms can be identified using the PKPD modeling approach. Depending on their mechanisms of action, an agent that works on targeting the cell wall may not work as well in Gram-negative bacteria. However, the PKPD model can support a quantitative distinction of a particular drug action across different species of bacteria.

Several limitations should be noted when interpreting PKPD relationship for antimicrobials. Bacteria–drug response is a dynamic process. The selective pressure from antimicrobial challenge often creates heterogeneous populations. As with an increased clinical usage of antimicrobial agents, the emergence of resistance strains has also been observed. This phenomenon sometimes requires a more complex PD structure. Various attempts to use PKPD models to capture the complex relationship have been published (Schmidt et al. 2008; Wu and Derendorf 2010). Another limitation is the translational understanding between in vitro and in vivo observations. In addition to a lack of an immune system in the in vitro setting, the amount of nutrients available for bacterial growth is different between the two

systems. Nonetheless, given the ethical reasons that limit the study of drug resistance in a clinical setting, the current PKPD modeling provides valuable drug response evaluations.

The complexity of the model greatly depends on the richness of the PD data as well as on the microbial behavior and adaptation. Over the years, clinical observations have consistently shown that microbes have the ability to evolve and adapt in order to overcome drug challenges. This warrants using multiple populations to describe the kill-curve behavior. A dosing regimen that targets both susceptible and resistant populations may be a key to prevent the emergence of antimicrobial resistance. Combination chemotherapy consisting of a β-lactam and a β-lactamase inhibitor is an example of therapeutic strategies that target both the susceptible and resistant populations. Complex modeling, such as those considering multiple populations, has been demonstrated by Campion (Campion et al. 2004) and Jumbe (Jumbe et al. 2003) and others.

Clinical Applications

As discussed previously, a relevant PKPD model requires the clinical profiles of unbound drug concentrations that can be simulated in an in vitro dynamic kill curve assay. This information can then be used to conduct in vitro PD experiments for PKPD modeling. Many studies of antimicrobial agents have been studied using this approach (Barbour et al. 2009a; de la Pena et al. 2004; Liu et al. 2005; Schmidt et al. 2009; Treyaprasert et al. 2007).

For a clinical trial, when the data are modeled with a population approach, the variants from the model can be used to predict therapeutic outcome using the Monte Carlo simulation (Drusano et al. 2000; Owens et al. 2005; Schuck and Derendorf 2005). Typically 1,000–10,000 subjects can be simulated to display the population distribution using model estimated parameters and population variance. For a time-dependent drug where the *in vivo* data show that the time of drug concentration above MIC for at least 50 % of dosing interval is required to achieve efficacy can now be assessed in a more meaningful population approach. If the objective of the study is to have 95 % of the population achieved a $T>\text{MIC}$ of at least 50 % of the dosing interval, Monte Carlo simulation can be used to assess dosing regimen needed to achieve the desirable outcome.

Conclusions

The dynamic kill-curve assay provides a superior model to evaluate PKPD relationship compared to the traditional MIC-based approach. Our current understanding of microbial resistance mechanisms infers that the microbial population is heterogeneous in nature. When challenged with an antimicrobial agent, a

subpopulation with resistant characteristics may be selected to prosper, resulting in an increase in MIC over the course of drug treatment. Hence, the traditional PKPD modeling approach based on the relationship of drug exposure and stationary MIC value measured at the start of treatment may be misleading. The complex relationship of microbes and drugs need to be revealed by measuring the microbial response to clinically relevant unbound drug concentrations in a dynamic kill-curve system. Mechanism-based PKPD models can then be constructed to gain insights into the time course of microbial dynamics. These models provide a quantitative approach to optimal drug candidate selection, hypothesis testing, and dose prediction and optimization. Incorporation of immune system into the PKPD modeling remains a challenge but it will be critical to aid the understanding of drug, microbe, and host immune relationship. Mechanism-based modeling approach with increasing complexity should be one of the essential tools to combat the increasing challenges of antimicrobial resistance.

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Chapter 5
Pharmacodynamic In Vitro Models to Determine the Effect of Antibiotics

Julia Michael, Aline Barth, Charlotte Kloft, and Hartmut Derendorf

Abstract Pharmacodynamic in vitro models are widely used to evaluate the antibiotic effect against microorganisms. With them it is possible to have full control of the drug profile (static or dynamic) within the system and to obtain the correspondent effect at a certain time period. Based on the experimental conditions and results a mathematical model describing the relationships between the effective concentration of the drug, the pharmacological effect, and time can be obtained. By means of simulations from the model it is possible to simulate and predict effects that optimize the treatment for defining the optimal dosage regimens. Once this information is available it can be used to support dose selection in a more rational manner.

As a prerequisite, it is important to evaluate the different pharmacodynamic models, considering the advantages and disadvantages, as well as the objective of the proposed study. Therefore this review focuses on the description of the general requirements for pharmacodynamic models providing an overview on up to now developed in vitro models.

Keywords In vitro models • PK • PD • Bacteria • Static models • Dynamic models

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Introduction

Why Do We Need In Vitro Models?

Pharmacokinetic data are relatively easy to obtain. They express what happens to the antibiotic drug in the body. But one may ask what the antibiotic does to the bacteria? And which concentration does one need for a period of time to achieve this effect?

Pharmacodynamic in vitro models can be used to analyze the effect profile of a drug on a special microbial agent. Information about the effect–concentration–relationship, effect–time relationship, different effects by various dosing regimens and the postantibiotic effect can be gathered out of these models.

The following chapter provides an overview on up to now developed pharmacodynamic in vitro models—that means models which are useful to investigate the effect of antimicrobial agents on bacteria.

The typical approach for antibiotic therapy is to maintain the antibiotic concentration above the minimum inhibitory concentration for a period of time (Li and Zhu 1997) or to increase the area under the concentration time curve. But these methods do not work in all cases.

For example, the antibacterial effect of bacteriostatic drugs like sulfonamides do not increase on the heightening of the drug, but depends on a correct level over an adequate period of time (Sanfilippo and Morvillo 1968). The problem is to find the right concentration, which is high enough to achieve an inhibitory effect but low enough to avoid side effects and useless high concentrations.

Of course in vitro models also have disadvantages and inadequacies. Bacteria have a different ultrastructure when grown in liquid medium differing from those in vivo and on hard surfaces (Lorian 1988). Differences in the structure and surface, respectively, seem to have a great influence on the outcome of antimicrobial tests (Brown et al. 1990) although these structures need not be identical with pathogenicity factors (Dalhoff 1985).

Therefore a detailed documentation is recommended.

Body fluids such as blood or urine are usually not appropriate in vitro media (Lorian 1988). Another deficiency of in vitro models is the mixture by stirring or shaking. Under in vitro conditions the cultures have to be stirred to avoid biofilm and incorrect distribution, but besides stirring provides aeration which yields in an increased growth (Lorian 1988).

This may lead to differences in growth over time between the “unstirred in vivo situation and in vitro”.

A great problem especially in static in vitro models is the depletion of nutrients, which leads to changes in cell size, envelope, lipopolysaccharides, phospholipids, proteins, fatty acids, and cation content. These adaptations to the environment influence the drug sensitivity (to cell envelope and membrane) that may change the amount of effective drug (Gilbert 1985).

That could be one reason for differences in vitro and in vivo activity of a drug.

Table 5.1 Requirements for in vitro models

Exchange of medium
Changing drug concentrations
Well mixture
Capture growing conditions
Capture inoculum size
Capture time dependent changes
Capture concentration dependent changes
Assess rate of bactericidal activity

Similarly, in vivo growth rates are difficult to assess out of static conditions, because on one hand static cultures show shorter generation times than in vivo; on the other hand in vivo cell numbers are also eliminated by immune defense and cell death (Gilbert 1985).

What Are the Minimum Requirements for In Vitro Models?

Pharmacodynamic in vitro models should exchange the medium following in vivo conditions. Too high or low flows make no sense if they falsify in vivo conditions. The in vitro models should also provide changing drug concentrations, which should accord to the pharmacokinetic behavior in vivo. Bacteria and medium ought to be well mixed to get a typical distribution of the samples. The record of inoculum size and growing conditions make the results reproducible. Table 5.1 shows a list of the requirements and regards for in vitro models.

Determine Terms

Some terms have to be described preliminarily to enable an exact comprehension of the text and avoid misunderstandings.

Static: Static systems are closed systems with no input and output of medium, biomass and secondary metabolites. Antibiotic concentrations remain constant at one initial level. These systems are also called closed systems (Gilbert 1985).

Dynamic: Dynamic systems (or open systems) allow an in- and output of medium and secondary metabolites (Gilbert 1985). The biomass may but need not change. Antibiotic concentrations can be kept constant or may vary.

Continuous culture: A continuous culture is defined by a constant volume in and out flowing in the model per time period. This is determinable for every time point; otherwise, it would be a stepwise dilution.

For this chapter only papers describing a new model or an innovation of an in vitro model have been taken into account. A classification for in vitro models has been developed (see Fig. 5.1) based on the structure Grasso (1985) described previously. In vitro models can be first characterized by the drug concentration. This can

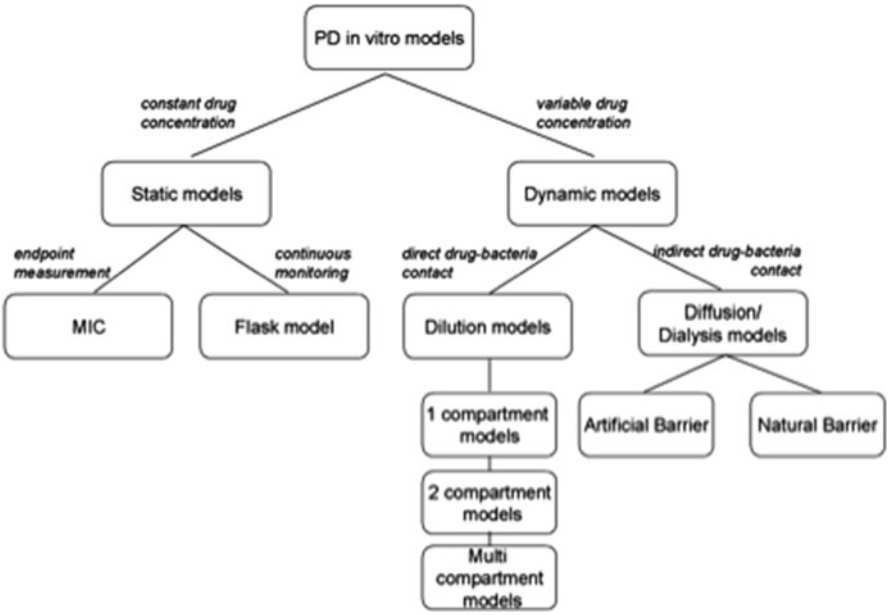

Fig. 5.1 Classification of in vitro models investigating the time-killing behavior (pharmacodynamics) of bacteria

be constant during the observation period—static models—or change like in dynamic models. A characteristic for these main basic models can be found in Table 5.2.

Static models result either in an endpoint measurement as it is the MIC or can be continuously monitored. Dynamic models are differentiated as well by the nature of drug-bacteria contact into dilution model with direct contact and into dialysis or diffusion models with indirect contact. Furthermore, the dilution models can be divided in one, two or multiple compartments through the use of different dilutions schemes.

Dialysis models are distinguished by the diffusion barrier in models with artificial barriers and such with a natural barrier.

Static Models

Static in vitro models are characterized by a constant drug level over the whole observation time (Vaddady et al. 2010). This is due to the same constant medium volume through the whole experiment (Vaddady et al. 2010).

Static in vitro models need only little effort in the laboratory and are easy to handle. They are useful to get an idea about the time-killing behavior (interaction) of antibiotics and bacteria.

Table 5.2 Characteristics of static and dynamic in vitro models

Characteristic/category	Static models	Dynamic models
System	Closed	Open
Drug concentration	Constant	Changing
Dilution of drug	–	+
Effort	Small	High
Dosing	Single	Single + multiple
Observation time	Max. 24 h depending on nutrient	Depending on the dosing regimen (up to 72 h or more)
Time-dependent changes	+ (not MIC)	+
Conc.-dependent changes	+	+
Persistence activity of antibiotics	–	Measurable
Medium volumes	Low	High
Nutrition conditions	No change of one given medium	Exchanging nutrient
Protein binding test pos.?	–/+ Not at all	+
No. of bacteria strains	One	Various
Bacteria cultures	One	One/multiple (see Table 5.1)
Bacteria loss	Non	Depending on the system
Dilution of bacteria	–	–/+ depends on the model

On the contrary to these economic aspects, static systems do not provide medium or drug exchange. That means they do not fulfill two major aspects of the in vivo conditions (in the body): exchange of nutrient and dilution of drug by a first-order kinetic. They are not useful for prolonged treatment studies, because of nutrients depletion and growth restriction. A release of secondary metabolites can appear which may inhibit growth (Gilbert 1985).

The technique of the minimum inhibitory concentration (MIC) provides an endpoint, whereas the flask models can also follow the time- and concentration-dependent changes. In the following these two basic static in vitro models shall be discussed.

MIC

The MIC, minimum inhibitory concentration, is the most common parameter in routine use which can be achieved out of a static model. It is well described in the European Pharmacopoeia (Ph.Eu.) (2012) and the United States Pharmacopeia (USP) (2012).

Its description is placed here, because the MIC is not only a parameter but also a method, which should be checked out here carefully.

For determining the MIC, an isolated organism is incubated with the antibiotic usually at 37 °C over 18 or 24 h [see USP (2012) or Ph.Eu. (2012)]. The minimum inhibitory concentration (MIC) is the lowest concentration, which shows no visible growth after that time period.

The MIC is a simple method and therefore suitable for routine use. It is a basic parameter for models which allow a good prediction of the potency of the drug–microorganism interaction (for detailed information see Mueller et al. 2004).

However, it is only an endpoint measurement and does not provide any information about the pharmacodynamic changes over this time period (Li and Zhu 1997; Vaddady et al. 2010). It means that time-dependent changes are not taken into account (Li and Zhu 1997). The rate of bactericidal activity stays unknown (Mueller et al. 2004). Also the persistence activity of the drug is not considered (Mueller et al. 2004), though this could be helpful in vivo where growth rates are slower and the immune defense works, too (Gilbert 1985).

Concentration-dependent changes cannot be revealed with this technique (Li and Zhu 1997; Mueller et al. 2004). Like all static methods, it permits only constant antibiotic concentrations, although in vivo they decline by a first-order elimination (Li and Zhu 1997).

Some aspects should be also taken into account, when estimating MIC values:

The outcome (MIC) depends on the size of test inoculum (Li and Zhu 1997), incubation period, and the used medium (Greenwood 1976). The MIC is mostly compared to plasma data, but these do not capture information about protein binding and tissue distribution (Mueller et al. 2004).

For these reasons other models were and shall be developed which allow a more precise modeling of in vivo conditions to understand the antibiotic–bacteria relationship better.

Flask Model

An easy model to detect the effect of antibiotics in a continuous manner was first described by Garett et al. (1966) (Fig. 5.2).

Bacteria were cultured in a flask with broth. The antibiotic is added to the flask and incubated with the bacteria. Samples are continuously taken to monitor the growth and dying behavior of the bacteria.

This "*flask model*" is a basic model for continuous monitoring of cultures. It provides information about the bacterial behavior under static drug levels without exchange of medium. Because of its easy application it is still today in use.

One disadvantage of the model is that it does not include an exchange of the medium which leads to a depletion of nutrient and limits the growth. When the maximum growth rate is achieved, the ratio of growth and death of bacteria stays constant. A static phase of bacterial growth is reached. A second disadvantage is the constant drug level, which is not typical in human.

The "flask model" was adapted by Scaglione et al. (1993), who used immune cells and human serum. This "*immune model*" shall simulate the bacterial time-killing behavior at the target side under drug and immune cell exposure. The method allows determining the antibiotic effect in- and outside the cells by measuring them

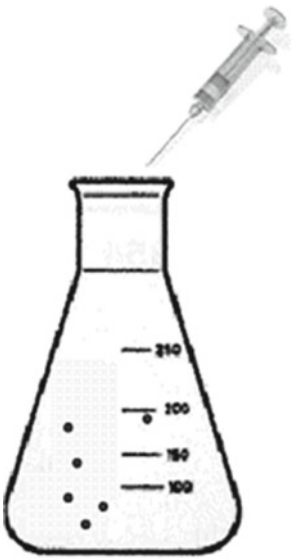

Fig. 5.2 Flask model, described by Garrett et al. (1966)

separately. It examines the penetration of drug into various cells and presents a realistic cell to fluid environment.

But it still suffers from the not changing medium and constant drug levels.

A very special static model was presented by Darouiche et al. (1994). They investigated the penetration of constant antibiotic concentrations through a biofilm (for biofilm models see also below (Vergeres and Blaser 1992)).

An orthopedic nut is incubated with slime-producing *Staphylococcus aureus*. After the nut has acquired a biofilm it is placed into a tube with a constant antibiotic concentration.

Even distribution is conferred by shaking.

Regrettably only one measurement of drug concentration and bacteria number is available from one orthopedic nut. For a statistical relevant conclusion does that mean an enormous labor input.

Dynamic Models

Dynamic models are so called, because of the changing drug concentration which is caused by changing medium (Gloede et al. 2010). Two basic principles were found in the literature: One, where drug, medium and bacteria are in the same compartment and the exchange takes place in this compartment by dilution, the other one, where the drug and medium first have to diffuse a barrier, for example, a membrane, before reaching and leaving the bacteria (Gloede et al. 2010).

Models working with the first principle are the so-called dilution models, whereas the others are diffusion or dialysis models (Grasso 1985; Gloede et al. 2010).

Dilution Models

Dilution models enable the study of prolonged treatments, which are not limited by nutrient depletion except the inflow itself is a limiting factor (Gilbert 1985). The stepwise or constant flow of medium provides variable drug concentrations. A drawback of these models are the large required volumes and the impairment of testing multiple cultures together (Blaser et al. 1985a). The in vivo half-life of the antibiotic is often is often simulated through the medium flow rate. For drugs with a long half-life this would mean not only very low flow rates but also low medium replacement and with it nutrient depletion. Also antibiotics with different half-lives cannot be used simultaneously (Blaser et al. 1985a), because of the inability of simulating this via flow.

In addition the early dilution models suffered from the dilution of bacterial inoculum, whereby a mathematical correction was necessary. A steady state of nutrient and cells is obtained in these open systems, where also biomass drops away (Gilbert 1985).

Next to the bacteria loss, also a bacteria back growth into the reservoir is common and falsifies the results.

One-Compartment Models

Dilution of antibiotics in the central compartment can be achieved by three ways: By adding fresh medium resulting in increased volume, by stepwise dilution (replacement of a defined volume with fresh medium in a stepwise fashion), and by continuous dilution.

Continuous Dilution with Increasing Volume

A simple method simulating dilution by increasing volume was described by Nishida et al. (1976) (Fig. 5.3). In a tube bacteria and antibiotic are incubated together. The human serum levels of the drug are achieved by increasing the volume periodically (stepwise).

Unfortunately the tubes are not shaken or stirred in this method, which means an incorrect mixture (distribution) of the bacteria may result in biofilms, aggregations, and false colony counts.

Nevertheless this "*tube method*" was adapted by Randolph et al. (1979) using 95 % human serum as medium. The influence of protein binding on the drug and the time-killing behavior of the bacteria was not investigated. Another drawback of the work is the difference in sample preparation between growth control and drug-containing tubes.

Fig. 5.3 Model described by Nishida (Nishida et al. 1976)

Sanfilippo and Morvillo (1968) described a one-compartment model, which should be the base for most models with continuous dilution (Fig. 5.4). The model consists of a series of flasks containing bacterial culture. Each flask is connected by rubber tubing with a flask in another series, which either contains sterile medium or medium with antibiotic. The fresh medium is pumped into the bacteria flask and therewith simulated increasing or constant drug levels. A pump with a timer is used to control the flow. The series of flasks enables simultaneous investigations. The model suffered from the inconstant rate diffusion. That leads to not exactly exponential drug concentrations (like in vivo).

Another model was developed several years before by O'Grady and Pennington (1966) (Fig. 5.5). Their "*urinary bladder model*" consists of a glass vessel with a tubular prolongation at the base. A metering pump provides a continuous flow of medium into the vessel. Since there is no outlet the volume of the vessel increases in this model, too.

A stirrer facilitates a well mixture of the bacteria in the medium. Continuous colony counting is performed by a photometer at the bottom of the vessel.

Another reported disadvantage is the accumulation of bacteria on the glass surface of the vessel. This leads to overestimated colony counts and biofilm progression.

The improvement of all these models is the ability to simulate in vivo drug concentrations (except the Sanfilippo model).

Though, all three models offer only one compartment specification so that drug–bacteria relationships with other conditions cannot be modeled. They also suffer from the increasing volume and therefore diluted bacteria counts which make a mathematical correction necessary.

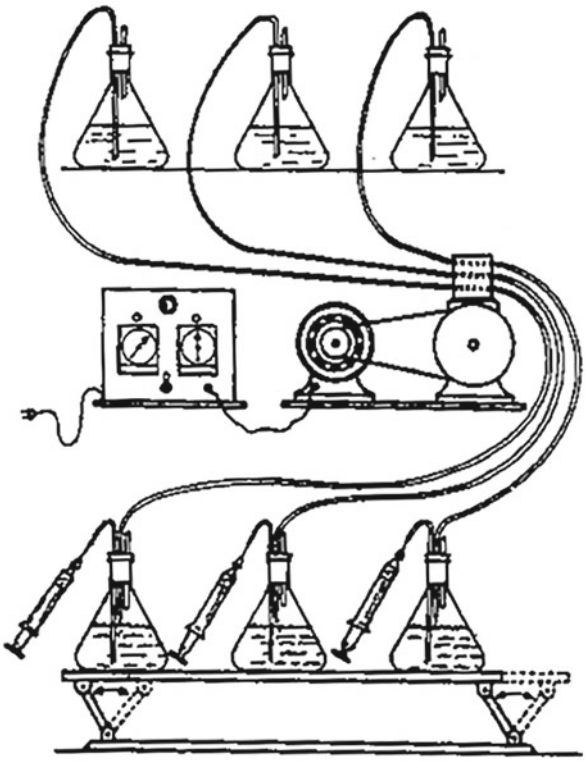

Fig. 5.4 Model by Sanfilippo and Morvillo (1968)

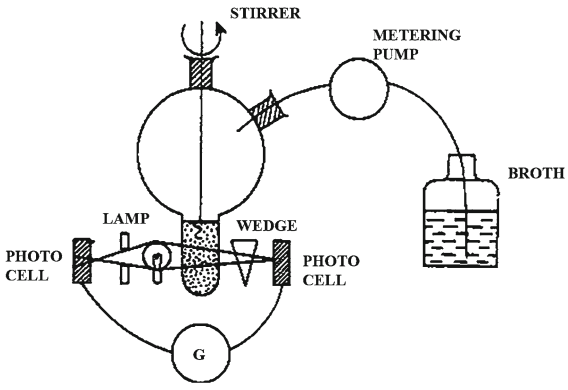

Fig. 5.5 Urinary bladder model by O'Grady and Pennington (1966)

Stepwise Dilution

An easily practicable model with a stepwise dilution and no increasing volume was first described by Nolting et al. (1996) (Fig. 5.6). The main compartment of the "*syringe model*" is a culture flask, where broth solution is taken off with a syringe

Fig. 5.6 Syringe model by Nolting et al. (1996)

at regular intervals and replaced by the same volume of fresh broth. A bacteria retaining filter is placed prior to the syringe plunger. Although this model does not offer a continuous dilution, and therewith not the same treatment of bacteria like in vivo, it is anyhow possible to achieve realistic results.

Haller (1982) offered another method to determine the antibiotic effect in a stepwise dilution model. He uses a Teflon-coated ultrafiltration cell which is filled up with medium and broth. After adding drug solution, a pressure with sterile air is applied. By this a constant volume is eluted per time period and discarded. An even volume of sterile medium was placed consistently on the cell after a constant time period.

While the antibiotic concentration changes not like in a continuous culture, the shortness of the intervals make these changes moderate and theoretical gradients could be simulated quite accurately (Haller 1982).

Continuous Dilution

The "*urinary bladder model*" was farther developed by O'Grady et al. (1973) (Fig. 5.7).

The presented model consists of a conical or cylindrical flask with a glass syringe barrel on the bottom welded to the flask. A photometer is adjusted at the syringe and over a complex pneumatic piston and a motor-driven camshaft timer, the flask fluid can enter the syringe periodically and a turbidity measurement is conducted.

But the important innovation is the periodically emptying of the fluid through an outlet.

Therewith a close simulation to the in vivo conditions of the bladder is achieved.

However, this model is only useful for simulation of infections in emptying compartments like the bladder. It is not applicable on tissue infections, for example, skin infections.

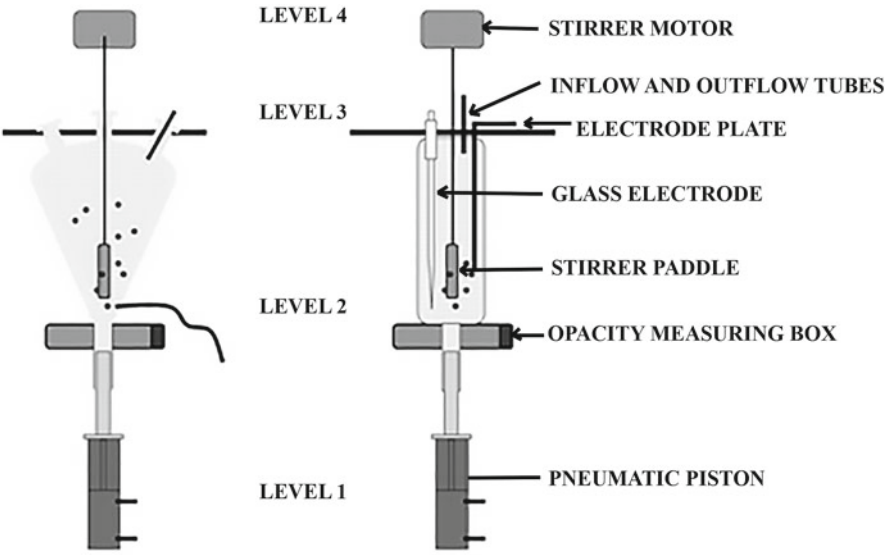

Fig. 5.7 Urinary bladder model. Adapted from O'Grady et al. (1973) with a conical (*left*) and the cylindrical flask (*right*)

Otaya et al. (1976) used culture tubes for their experiments. An air pump brings fresh medium from a reservoir into the tube, where it flows out at the same rate. Reported problems were the pressure regulation, which impedes the laboratory use, and the dilution of bacteria, which makes a mathematically correction again necessary (Grasso 1985).

A decisive improvement of the dilution models was the one-compartment open model developed by Grasso et al. (1978) (Fig. 5.8).

A flask containing bacteria and drug is connected with a two-hole rubber stopper with glass and Tygon tubing to a reservoir. Fresh medium is pumped by a peristaltic pump from the reservoir into the flask (as already known from Sanfilippo and Morvillo (1968)). A vessel collects the outgoing fluid. Samples are taken out of the central compartment. A magnetic stirrer ensures a well distribution. This model enables simulating intravenous bolus administration.

To simulate extravascular administration (first order absorption), Grasso added a second flask (compartment) prior to the main compartment.

In both Grasso models the bacteria are diluted by the incoming medium and flow out with the outgoing medium, which again makes a mathematical correction of the counted bacteria obligate.

The "*Grasso model*" was adapted by several groups (see Gerber et al. 1982; Satta et al. 1988; Firsov et al. 1985, 1988).

Bergan et al. (1980) used a second peristaltic pump for the outflow (Fig. 5.9).

Afterwards it was farther utilized by Ledergerber et al. (1985), who used a computer controlling three pumps sets parallel and fixed a turbidity cell at the glass flask (Fig. 5.10).

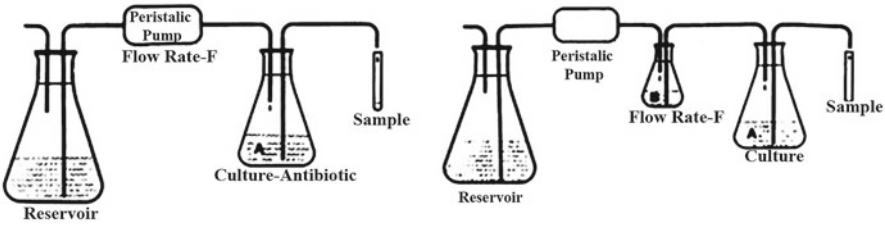

Fig. 5.8 Grasso et al (1978) models for monoexponential decrease of antibiotic (*left* side) and biexponential time curve like after extravascular administration (*right* side)

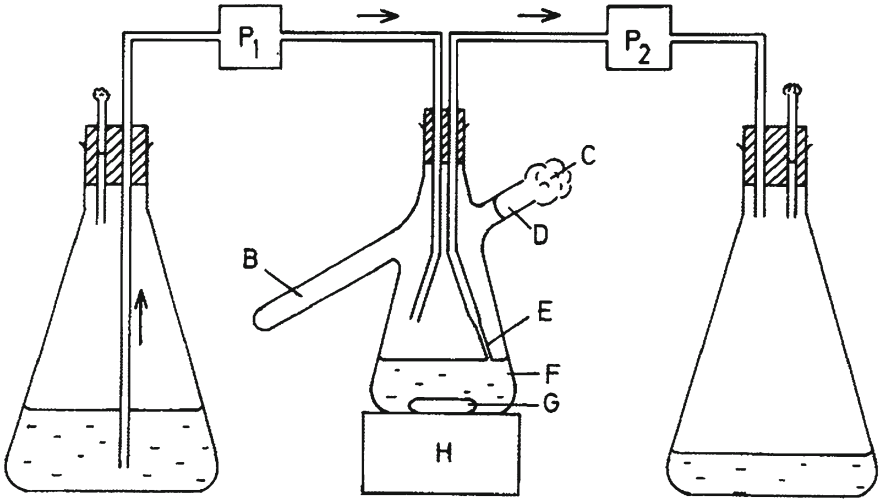

Fig. 5.9 Model by Bergan et al. (1980). "P1" and "P2" correspond to the pumps,"A" to the reservoir with sterile medium, "B" to the photometric tube, "C" to cotton plug, "D" to sampling tube, "E" to outlet tubing, "F" to the culture compartment, "G" to the magnetic bar, "H" to the magnetic stirrer and "I" to waste flask

Schneider et al. (1982) avoided the bacteria loss in the Grasso model (Fig. 5.11). They placed a filter in front of the outlet, which's place had changed to the bottom of the chamber. A water-jacket surrounds the whole chamber to keep the temperature constant inside. The model also uses a turbidity cell for online bacteria counting.

Also Shah (1980, 1981) improved the original Grasso model while using a microglass filter to retain bacteria.

Another solution for the continuous flow without increasing volume or bacteria loss was found by Greenwood and Tupper (1982) (Fig. 5.12).

The model presents two small chambers which are separated by a cellulose acetate filter membrane. The upper chamber contains the bacteria and a magnetic stirrer keeps the membrane pores free. The fresh medium enters the upper chamber, passes

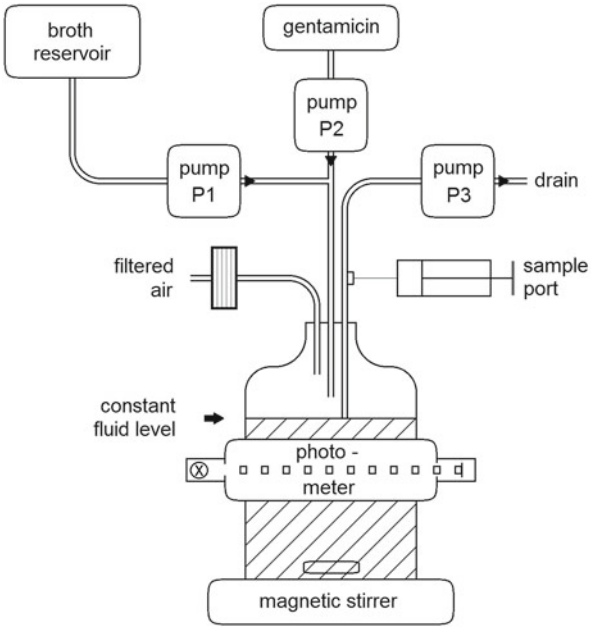

Fig. 5.10 Model by Ledergerber et al. (1985)

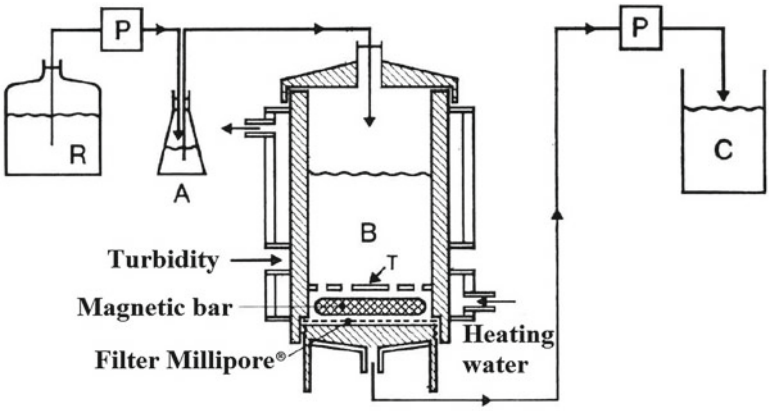

Fig. 5.11 Model by Schneider et al. (1982). "R" corresponds to the medium reservoir, "A" to the flask containing the antibiotic, "B" to the compartment containing the bacteria, "P" to pumps, "C" to waste and "T" to the baffle against turbulence

the membrane to the lower chamber, and leaves the vessel there. The drug can be placed in the upper chamber and washed out with a first-order kinetic by the flowing medium.

Although this seems an appropriate approach, drug concentrations were not exactly the same like in vivo, because the concentration did not change continuously, but at least corresponded at appropriate intervals (Grasso 1985).

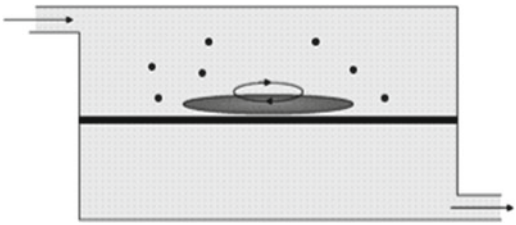

Fig. 5.12 Model described by Greenwood and Tupper (1982)

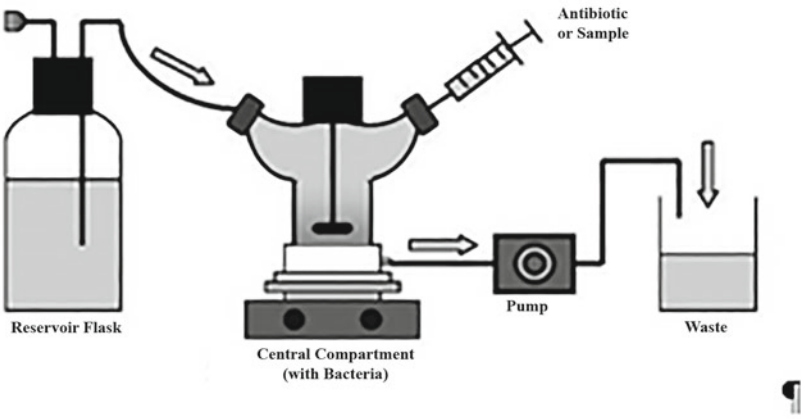

Fig. 5.13 Model described by Lowdin (1996)

A different solution for overcoming the bacteria loss and the increasing volume was found by Lowdin et al. (1996) based on the Grasso model (Fig. 5.13).

For this model the base of the culture vessel is separated from the remaining flask. A new bottom is added with an outlet and a perforated metal support, on which a filter membrane and a pre-filter are adjusted. Above the membrane a magnetic stirrer is placed to prevent membrane blockage. One of the side arms of the flask has a silicon membrane to enable repeated sampling. The other arm has a thin plastic tubing where fresh medium can enter the central compartment. A pump assures the constant flow of medium.

Although this model seems to solve all problems, some new disadvantages appeared. One thing is the blockage of membrane, which has not been completely avoided by the magnetic stirrer. The other hard part is to clamp the two parts of the flask closely together, when the system is filled up with medium. Furthermore a "back growth" of bacteria from the central compartment (flask) into the reservoir with fresh medium is possible. Lignell et al. (2007) modified this model to study the pharmacodynamics of antifungal agents.

Budha et al. (2009) adapted a compartment model for the use with slow growing microorganisms such as mycobacterium, as shown in Fig. 5.14. They added a hollow steel tube containing a filter holder in the bottom and therefore the media is removed from the superior part without the presence of microorganisms, reducing

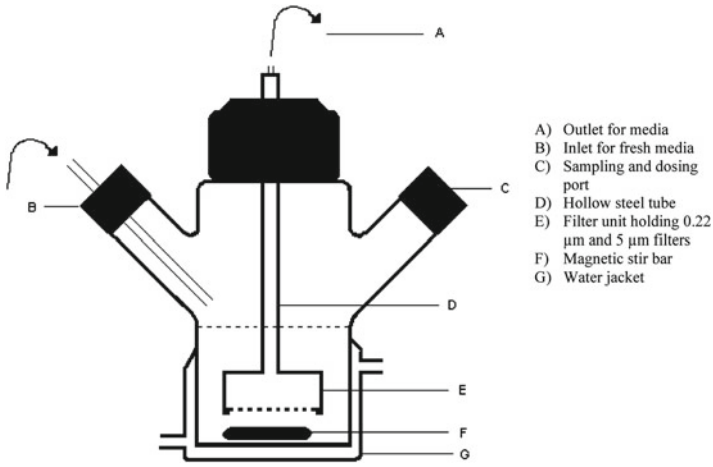

Fig. 5.14 Model by Budha et al. (2009)

some of the problems cited above, as membrane pore blockage. Fresh media is replaced at the same rate in one of the arms (B), keeping the fluid level in the central compartment constant. The media flow in both directions is driven by peristaltic pumps. The system contains a water jacket to keep the temperature constant at 37 °C and a magnetic stirrer to keep the media homogeneous.

Two-Compartment Models

A two-compartment model was first described by Murakawa et al. (1980) (Fig. 5.15). Two vessels, the central and peripheral compartment, are connected by an air-tight silicon tubing. The central compartment keeps the bacteria and the drug. A pump forwards the medium into the central compartment. From there a second pump brings the fluid with a constant rate into the peripheral compartment. Magnetic stirrers in both compartments ensure the distribution of drug and bacteria. When the experiment starts the peripheral compartment is drug free. Samples are taken out of the central compartment.

In this model the bacteria do not only get lost into the waste, they are also distributed into the second compartment. During a short time they are highly distributed and diluted. They can colonize the central and peripheral compartment, the reservoir, and the waste, which complicates a mathematical correction.

Because bacteria are mostly situated in the extravascular space and not in the compartment where the drug is administered, another approach with bacteria in the peripheral compartment without the ability to leave it would be more interesting.

A new model was created by Meletiadis et al. (2012) and it was soon used in additional studies by the same research group (Al-Saigh et al, 2012) (Fig. 5.16).

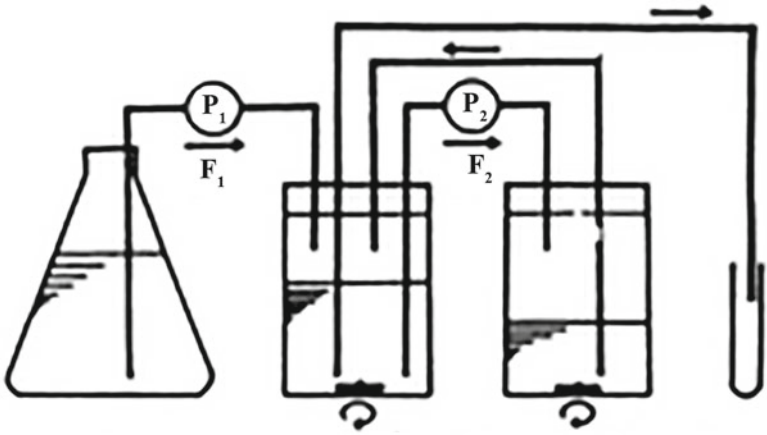

Fig. 5.15 Model by Murakawa et al. (1980). P1 and P2 correspond to the pumps, and F1 and F2 to the flow rates generated by these pumps

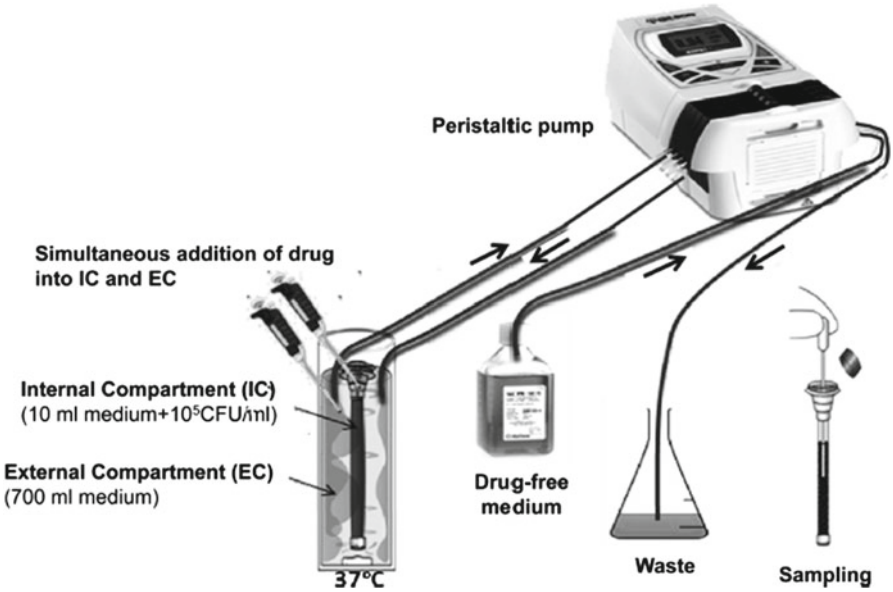

Fig. 5.16 Model by Meletiadis et al. (2012)

The model is composed by both an internal (IC) and one external compartment (EC). The IC is made of cellulose membrane with a specific cut-off that does not allow the passage of large molecules. It contains media and inoculum, while the EC contains only the media. The EC is a beaker and it is kept at controlled temperature and under constant stirring. Antibiotics are placed in both compartments in order to

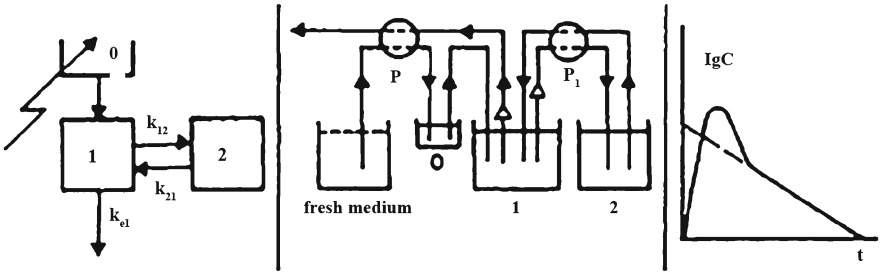

Fig. 5.17 Model by Navashin et al. (1989). *Left*: representation of a two compartment model with the micro-constants K12, K21 and kel. Center: two compartments in vitro system where P and P1 represent the pumps. Right: plot of the logarithm of the concentration (lgC) versus the time (t) for a two compartments model

allow a faster equilibrium. Fresh media is continuously pumped into and out of the external compartment, diluting the antimicrobial content, simulating its half-life.

Multi-compartment Models

Principle ideas for multi-compartment models were presented by Rowe and Morozowich (1969). They showed the assembling of the in vitro model and the respective equations for the drug concentrations.

For the multi-compartment model presented by Navashin et al. (1989) several vessels were connected (Fig. 5.17). The number of vessels depends on the modeled compartment number. The vessels are connected by syringe needles fitted with silicone pipes.

One vessel is assumed as absorption site (vessel 0). The drug application can happen there or in the central compartment. Fresh medium is pumped from the reservoir into the central compartment (vessel 1) where also the elimination takes place. Other pumps assure the transport of medium from the central compartment (vessel 1) into the other compartments (vessel 2 or *x*) and back. The bacteria are in the central compartment (vessel 1); a biophotometer cell allows the online turbidity measurement of the cells. The bacteria loss is prevented by a filtration unit. The incoming fresh medium is running over the filtration unit, so that this should not be blocked.

The model offers a lot of possibilities, if some aspects are repeatable. One is the avoided membrane blockage by rinsing with fresh medium. Another aspect is the possible back growth of bacteria into the reservoir. Not mentioned are stirrers which afford an even distribution of bacteria and medium. On this account the model is serious for multi-compartmental use, but still needs some improvements.

Dialysis or Diffusion Models

Dialysis or diffusion models are by their nature always two-compartment models. They consist of a central and a peripheral compartment, separated by a membrane, which can be passed by the drug and medium, but not by the bacteria. The medium in- and output occurs in the central compartment. The exchange of medium in the peripheral compartment, where the bacteria are, happens indirectly by diffusion. The antibiotic can enter the central compartment on different ways (simulating i.v. bolus or extravascular administration, for example). There it takes the same way as the medium and reaches the peripheral compartment by passive diffusion.

Dialysis models do not have the problem of bacteria dilution, but of accumulation of bacteria on membranes (Blaser et al. 1985a), which might lead to membrane blockage, interrupted diffusion, and no homogenous bacteria treatment.

In the following, dialysis models shall be discussed by the nature of their membrane. In the past artificial membranes known from hemodialysis were used. The shape of artificial membranes changed from a plane area (face) to hollow fibers.

But in the literature also models with a natural barrier or membrane can be found. The antibiotic first has to pass a barrier before reaching the bacteria. This natural barrier can consist of tissue cells, fibrin, or slime as described below.

Since this drug–bacteria interaction has the same character as mentioned before for the artificial membrane, they are grouped together.

This classification of some models might be uncommon, but comprehensible if one looks at the way of action.

Artificial Barrier

In the "*tube dialysis model*" of Al-Asadi et al. (1979) a cellulose acetate membrane separates two tubes with antibiotic in one and bacteria in the other tube (Fig. 5.18). The system is clamped together tightly. Al-Asadi et al. (1979) first allowed the drug to enter the bacteria tube by diffusion. To reverse this effect a peristaltic pump forces the medium through a syringe needle in the rubber bung into the bacteria-containing tube, a second pump guarantees the outlet and a continuous flow. A Teflon-coated magnetic stirrer in each tube ensures a well distribution.

Nevertheless bacteria block the membrane pores and therefore impede the diffusion of antibiotic. Furthermore it is not clear why the author flushed the medium from the bacteria tube to the drug tube and not otherwise around, although this might show a more exact first-order elimination of the drug.

Drugeon et al. (1979, 1982) described a vessel/chamber with bacteria and antibiotic (Fig. 5.19). Fresh medium is pumped into a dialysis unit where it can diffuse into the chamber system and also drug and exhausted medium can pass out. The surface offered for the dialysis process is very small in this model. However, the principle of this simple "*chamber model*" was adapted by different groups (see below).

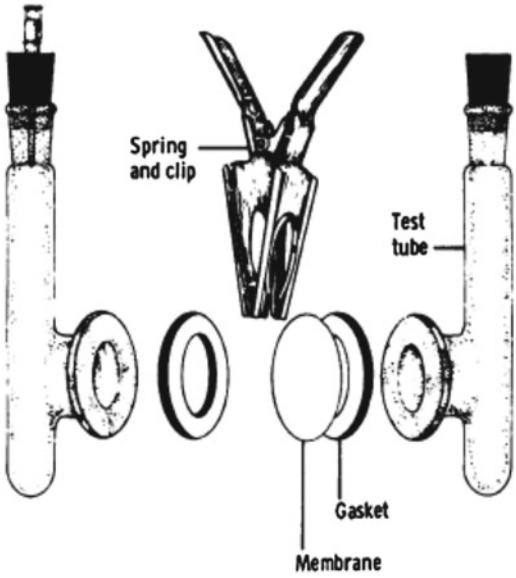

Fig. 5.18 Model by Al-Asadi et al. (1979)

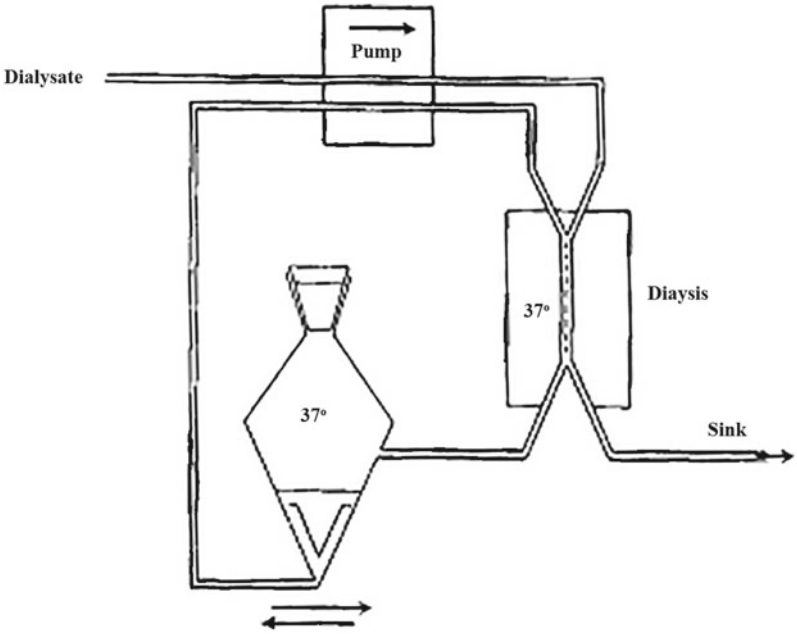

Fig. 5.19 Model by Drugeon et al. (1979, 1982)

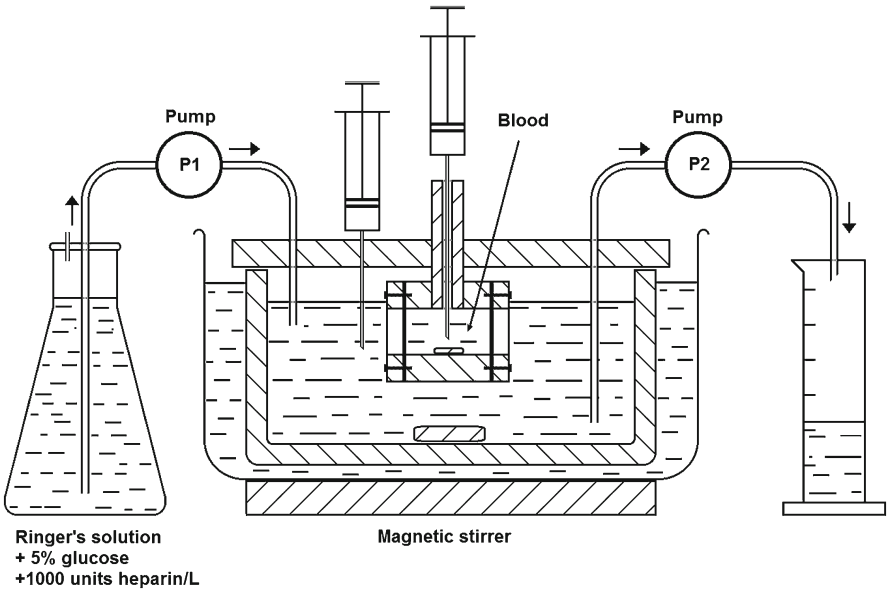

Fig. 5.20 Model by Shah (1985)

Shah (1985) changed the surface of exchange by putting the bacteria chamber into a medium jar (Fig. 5.20). The inner chamber consists of Plexiglas with changeable membrane filters at both ends. The exchange of medium and drug can take place at both sides.

Fresh medium is pumped from a reservoir into the outer chamber and from there out in a waste. The continuous flow is controlled. Magnetic stirrers assure a homogenous medium in the inner and outer chamber.

However, a membrane blockage by growing bacteria can easily impede the diffusion.

The same kind of model was also used by Garrison et al. (1990). The inner chamber is a hollow t-tube with an inert polycarbonate membrane.

Vance-Bryan et al. (1992) estimated and changed the Shah model to the exact ratio of the surface membrane area to the volume of the peripheral compartment.

In all these "chamber models" a membrane blockage by growing bacteria can easily impair the diffusion. Other advances were made, where the inner chamber and its surface were changed.

Zinner et al. (1981) (Fig. 5.21) replaced the original outer chamber with artificial capillaries. Hundred fifty polysulfone fibers are surrounded by a chamber and their lumina are connected to a perfusion tube and a reservoir. The hollow fibers (which give the model's name "*hollow fiber model*") operate as the central compartment, where drug and medium rinse through. The chamber, that means the part outside of the capillaries, contains the bacteria and forms the peripheral compartment.

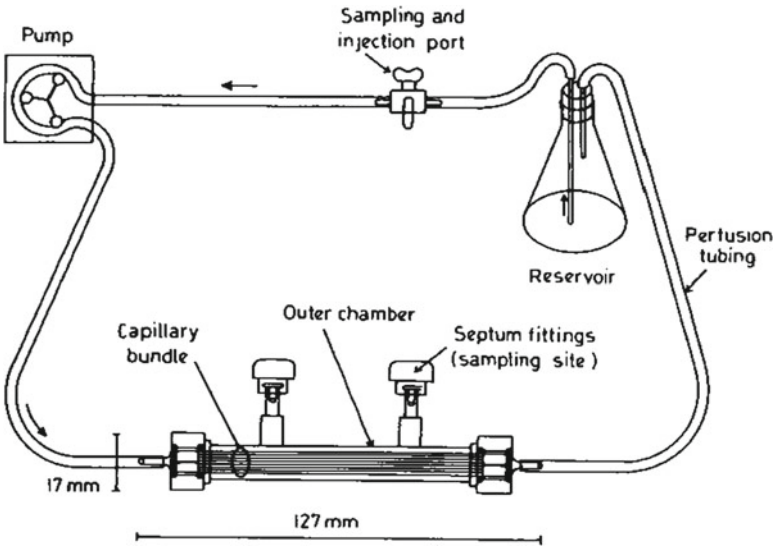

Fig. 5.21 Hollow fiber model by Zinner et al. (1981)

The capillaries are continuously flushed with medium by a pump. The connecting tube includes a stopcock as sampling and injection port. Medium and drug diffuse from inside the capillaries into the chamber and back. Bacteria stay outside the chamber.

A drawback is the standing medium in the peripheral compartment (the chamber) and so bacteria can adhere on the capillaries and other surface. A diffusion blockage or inhomogeneous samples are the results.

Blaser et al. (1985a, b) picked up the hollow fiber model and inserted a second or multiple bacteria compartments. Each bacteria compartment (peripheral compartment) consists of a polycarbonate chamber where 150 artificial Vitafiber® capillaries run through. The capillaries are of hollow polysulfone fibers connected to the central compartment.

A magnetic stirrer in the main vessel of the central compartment assures distribution.

The continuous dilution of the capillaries is done by a pump. Fresh medium is supplied by a reservoir and pumped through the capillaries, where exchange of medium and drug could take place. Used medium is eliminated in another vessel.

Via an infusion pump various drug administrations and kinetics could be simulated.

A special sampling port at the peripheral compartment is installed.

One reason for more peripheral compartments is the ability to investigate different cultures simultaneously like they appear in vivo. The apparatus seems to be less laborious.

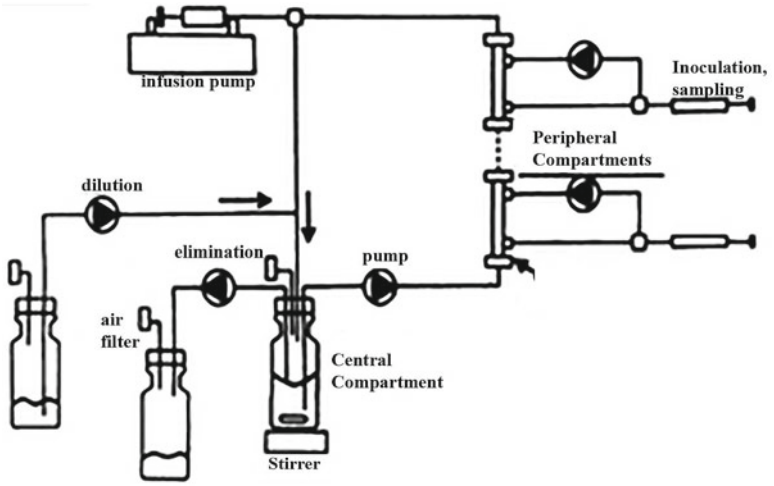

Fig. 5.22 Model by Blaser et al. (1985b)

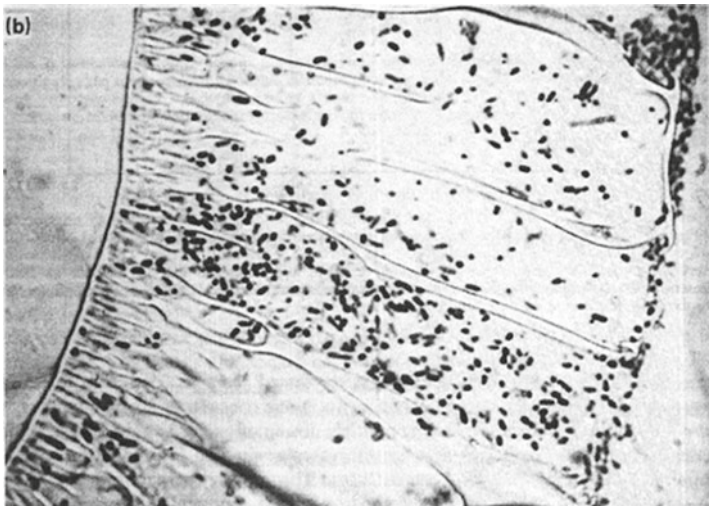

Fig. 5.23 Adherence of bacteria along the capillary (Blaser et al. 1985a)

Though as mentioned before, the authors of this study reported a bacteria cluster along the outside of the capillaries and also within the pores of the capillary wall (Figs. 5.21, 5.22, and 5.23).

That means improvements preventing the adherence of bacteria are necessary; otherwise, the diffusion might be blocked again, the bacteria in the peripheral compartment are not homogenously treated, and samples do not reflect the correct bacterial count.

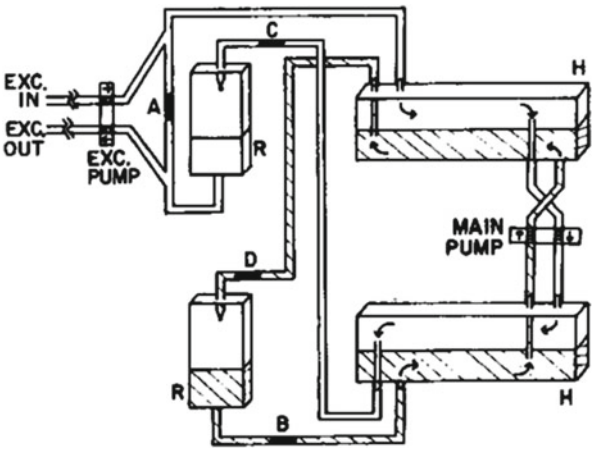

Fig. 5.24 Hemodialyser model by Toothaker et al. (1982)

Another very complex system based on the hollow fiber model was explained by Ba et al. (2001). They inserted liquid flow meters, a computer control for the pumps, as well as a fraction collector to make the process more accurate.

But in the author's opinion, the problem of colonization of the capillaries was still not solved.

Drusano and colleagues have been conducting different studies with the hollow fiber system, including the use of multiple drugs and the use of slow-growing microorganisms (Nicasio et al. 2012; Drusano et al. 2010; Gumbo et al. 2004). Additionally, a semi-automated two compartment hollow fiber system was developed by Wang et al. (2008). Some of the advantages of this model include a reduction in the amount of bench work and a minimization of the contamination related to manual sampling and environment exposure. Therefore the hollow fiber system proved to be useful and reliable to perform time–kill curves with microorganisms in order to evaluate the drug and microorganism interaction, mimicking the drug concentration in the human body over time.

A "*hemodialyser model*" was reported by Toothaker et al. (1982) (Fig. 5.24). They used a chamber, which is separated by a hemodialyser in two parts. The upper part contains fresh medium and the antibiotic, the lower part contains the bacteria. For increase membrane surface reasons two hemodialyser vessels are connected in the model. The lower parts of the chambers and a reservoir compose one closed circulating system. The upper parts, a reservoir with fresh medium and a waste arrange an open circulating system. The flow directions of the closed bacteria and the open medium system are contrary. The antibiotic dissolved in broth is pumped to the central compartment (the upper part of the chamber) and diffuses there into the lower part and back.

The continuous flushing compensates the magnetic stirrer found in the other models to provide distribution. The question is again if the membranes are blocked by the bacteria.

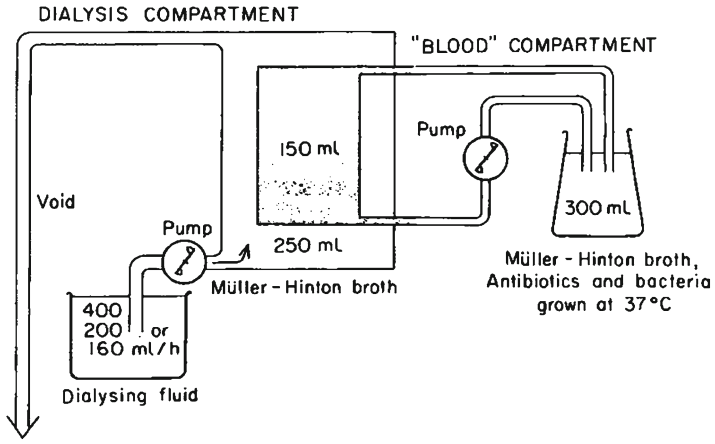

Fig. 5.25 Artificial kidney model by Guggenbichler et al. (1985)

The last different approach described for dialysis models with an artificial barrier, which should be discussed here, was the "*artificial kidney model*" by Guggenbichler et al. (1985) (Fig. 5.25). The dialysing unit consists of an artificial kidney of regenerated cellulose. It presents the peripheral compartment with the bacteria. The kidney is connected to a reservoir as a closed system. A pump cares for a continuous flow.

The peripheral compartment is surrounded by a chamber representing the central (or blood) compartment. A pump transports fresh medium from a reservoir through the chamber, where drug and medium exchange take place, to a waste.

The model is similar to the Toothaker "hemodialyser model" with its one closed and one open circle. It also has the same idea as Shah's model, who placed the bacteria compartment inside the central compartment.

The artificial kidney model was applied by König et al. (1986a, b).

Natural Barrier

In the dialysis models with the here called "natural barrier" a membrane of natural origin like cells or slime serves as separation between bacteria in the peripheral and drug and fresh medium in the central compartment.

The first model where cells have to be passed by the drug to get to the bacteria was a "*tissue culture model*" described by Haller (1985) (Fig. 5.26). Although the model was not mentioned for studying the drug effect on bacteria, it established the basic idea of cells as barrier.

Vero (monkey kidney) cells are grown on a dialysis ultrafilter of cellulose nitrate until a continuous layer appears. This membrane is assembled in a glass slide with

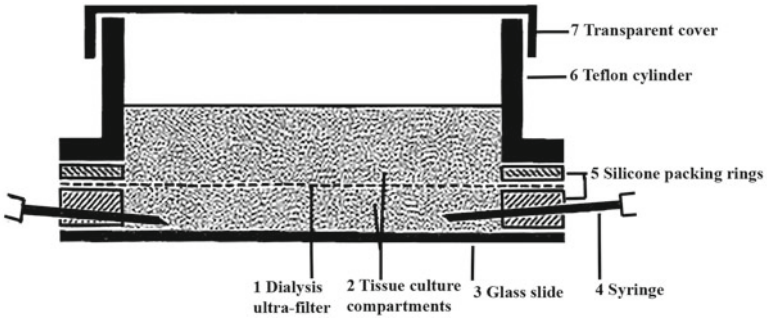

Fig. 5.26 Tissue culture model by Haller (1985)

a Teflon cylinder and a covering so that it separates it in an upper and a lower part. Syringes work as in- and outlet. The antibiotic administered in the lower part of the chamber can pass the cells to the upper part.

The model was suggested to investigate the penetration of drug through the cells. It offers an in vitro simulation of antibiotic concentrations at the target site. It is also possible to check protein binding with that model by comparing the drug in the upper, in the lower chamber part, and in the cells, but it is complicated. The medium in the model was not stirred or otherwise mixed which means homogenous distribution is not guaranteed. The volume of the chamber was very small, which has to be changed for further investigations to realize enough samples.

Although the model was originally not mentioned to investigate drug–bacteria effects, it was used and adapted by numerous groups (Hulten et al. 1996; Birkness et al. 1995, 1999; Shaw et al. 1987; Shaw and Falkow 1988) who investigated, not only what part of the drug penetrates the cells and effects the bacteria but also what part of the drug permeates the cell membrane and is effective against intracellular microbial agents.

A further step for the implementation of tissue cultures in pharmacodynamic drug–bacteria interaction models was done by Hulten et al. (1996) (Fig. 5.27). The model consists of a glass chamber with two exits and a metal rack for Falcon cell culture inserts as central compartment. The Falcon cell culture inserts have a cyclopore polyethylene terephthalate tack-etched membrane. The cells were cultured in this inserts. Since Helicobacter pylori, an intracellular agent, was investigated in the study, the cell itself presents the peripheral compartment, where the drug has to penetrate in and effect. Fresh medium is pumped through the glass chamber, where a magnetic stirrer assures even drug concentrations.

Bacteria and drug samples can be taken after destruction of the cells. The intensive sample preparation and the reduced number of samples are disadvantageous for this otherwise interesting model.

The "*fibrin clot model*" of McGrath et al. (1994) (Fig. 5.28) remembers again of the model by Shah (1985) (see above). Bacteria are suspended in a mixture of human cryoprecipitate antihemolytic factor, monofilament line, bovine thrombin

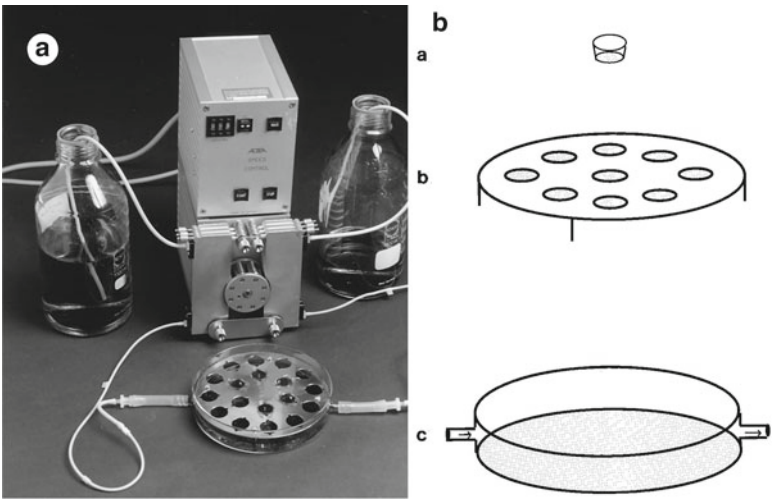

Fig. 5.27 Model by Hulten et al. (1996)

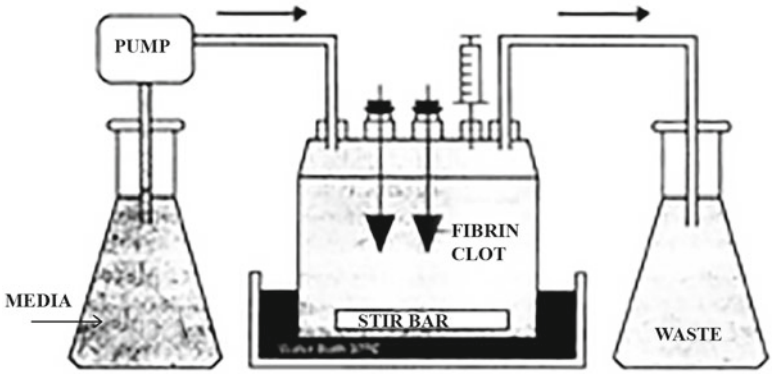

Fig. 5.28 Fibrin clot model by McGrath et al. (1994)

and sterile calcium chloride. A fibrin clot including the bacteria appears and is put into a chamber with medium. The fibrin clot presents the peripheral compartment. The chamber, which is the central compartment, is flushed continuously with fresh medium and antibiotic. The drug must pass the fibrin clot to attain the bacteria. For measuring the drug concentration and bacteria number in the clot, the whole fibrin clot has to be taken off, which reduces the number of possible samples. Also the laborious sample preparation limits the method.

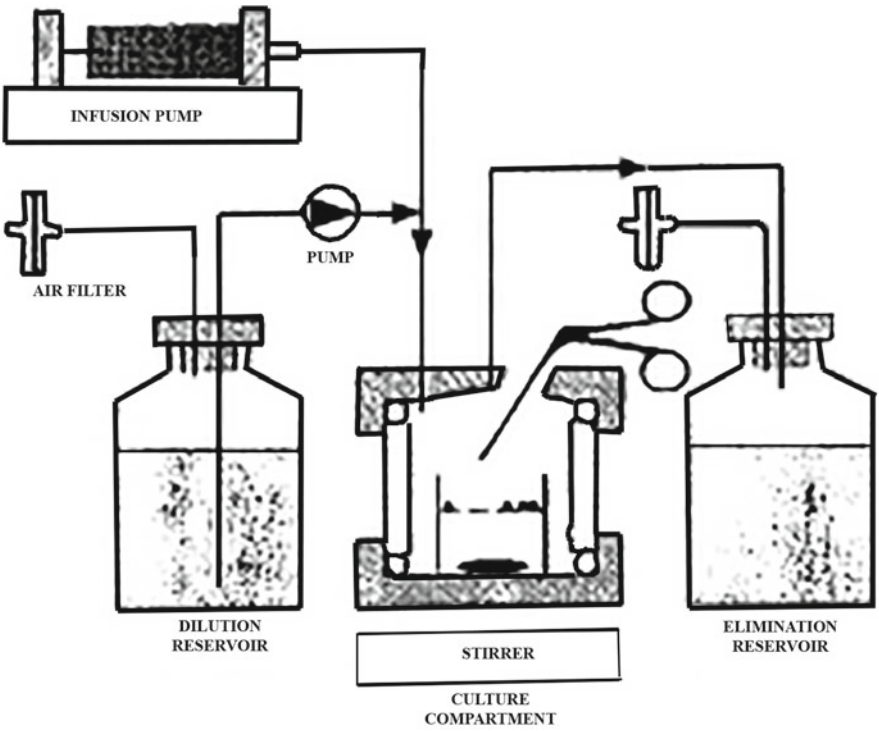

Fig. 5.29 Biofilm model by Vergeres and Blaser (1992)

Another natural barrier in dialysis models can be bacteria slime. Vergeres and Blaser (1992) used slime-producing *Pseudomonas aeruginosa* and *Staphylococcus aureus* in their experiments (Fig. 5.29). Bacteria are cultured on glass beads to achieve a biofilm which has to be diffused by the antibiotic. The glass beads stay in a wire cage in a chamber. Fresh medium is pumped out of a reservoir into the chamber and from there into the waste. Drug concentrations can be easily simulated with an infusion pump. The assembling is nearly the same like in the Grasso model except that the drug has to diffuse through the slime before it gets in contact with the bacteria. This diffusion process classifies the model as a dialysis or diffusion model. The difference to the model described by Darouiche et al. (1994) is the continuously changing drug concentration in the central compartment. Also in this model as well as in the model by Hulten and McGrath the number of samples is reduced by the number of glass beads. Since the bacteria have to produce slime, the model fits only a few strains.

Dialysis models with natural barrier have been designed for special demands. This makes them effective for their purpose but also only for this purpose. Anyhow, the problem of adhering bacteria can be neglected in these models.

Conclusion

Several in vitro models for studying the pharmacodynamic interaction between drug and bacteria have been investigated. Some of the models have become obsolete, others have been improved and are still in use.

Specialized models like the natural barrier dialysis models offer good results for the investigated problem. But they have also only limited use.

What is still missing, is a practicable model for various purposes. Serious problems seem to be the adherence of bacteria to filters, a back growth into the reservoir and a manageable glass body.

Static systems can be used for quick determination of the time-killing behavior. Dynamic models provide more information under changing drug concentrations and their killing effect.

Clinically appearing pharmacokinetic profiles should be investigated in such Pharmacodynamics in vitro models to investigate the relationship between drug concentration and effect. Using this relationship, the antimicrobial chemotherapy and the dosing regimen can be improved.

Of course, in vitro models cannot provide all in vivo conditions even because not all conditions for antibiotics and bacteria in vivo may be known.

To support an antimicrobial chemotherapy with a optimum dose response, all factors, drug, patient, and infecting agent, must be taken into account.

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Chapter 6
Population Pharmacokinetic–Pharmacodynamic (PK/PD) Modeling of Anti-infective Agents and Its Applications to Individualized Therapy

Alexander A. Vinks

Abstract This chapter describes the general principles of population pharmacokinetic–pharmacodynamic (PK/PD) modeling and its application to optimization of antibiotic dosing. Parametric and nonparametric pharmacokinetic modeling approaches are discussed. Population modeling will identify central tendency of PK/PD parameter estimates, quantify between and within patient variability, and identify clinically useful covariates. Population modeling has become an important component of quantitative model-based drug development. In addition, PK/PD models can be important extensions of therapeutic drug management in infectious diseases. The concept of population model-based individualized antimicrobial therapy is described. With the availability of population modeling for obtaining PK parameter estimates, the focus has shifted to quantifying the antimicrobial effect and linking kinetics to drug effects. Mathematical models that link drug exposure (PK) with PD indices that correlate with microbiological and clinical outcomes will provide us with a better rationale for proper dose selection of antimicrobial therapy in different patient populations.

Keywords Population pharmacokinetics • Pharmacokinetic–pharmacodynamic modeling • Antibiotics • Individualized dosing • Bayesian adaptive control

Introduction

Rational antimicrobial therapy is dependent upon a basic understanding of the way patients handle antibiotics (pharmacokinetics; "what the body does to the drug") and their response to specific drug effects on microorganisms and the host immune

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system (pharmacodynamics; "what the drug does to the body and the microorganism") (Benet 1984; Vinks 2002).

Advances in the field of pharmacokinetics, including the development of specific and sensitive methods for concentration measurements in biological fluids, have expanded our understanding of the time course of drug concentrations in plasma and the processes (absorption, distribution, metabolism and excretion, ADME) that influence the amount of drug that reaches the target in the circulation, organs, and tissues. While imperfect, there is almost always a better relationship between the action of a given drug and its concentration in the blood or at its effect site(s) than between the dose of the drug given and the effect. However, the time course of drug concentrations in the body cannot in itself predict the time course or magnitude of antimicrobial effect. In recent years great progress has been made in the characterization of antimicrobial pharmacodynamics. Specific PK/PD indices have been identified which are of major importance for efficacy and for the prevention of the emergence of resistance (Craig 1998, 2003; Ambrose et al. 2007) [Chaps. 1 and 2—Craig]. These PK/PD indices represent the full time course of in vitro and in vivo time–kill profiles of different drug classes. In addition, mechanism-based models have been developed that link PK/PD and allow the prediction of the dose–exposure–effect relationship of antimicrobial agents (Vinks 2002) [Chaps. 4 and 7; Derendorf and Tam].

Population Pharmacokinetic Modeling

The purpose of population pharmacokinetics is to describe the statistical distribution of pharmacokinetic parameter estimates and identify potential sources of intra- and interindividual variability among patients in the population under study. In contrast to traditional approaches, population modeling allows a detailed analysis of variability including to what extend demographic parameters (e.g., age, body size parameters such as weight) and (patho)physiologic conditions of excretory and metabolic functions (e.g., as reflected by creatinine clearance) are predictive of PK using sparse data collected from a large number of patients.

The traditional method of pharmacokinetic data analysis uses a two-stage approach and requires multiple serial sampling over time in few subjects. A structural model is fitted to the each individual dataset using a least squares algorithm that minimizes the difference between observed and the model predicted concentrations. For reasons of simplicity, the assumption is made that differences between the observed and predicted concentrations are caused by random error. With this traditional type of analysis, PK parameter estimates are found for each subject and typically summarized as mean and standard deviation across individuals. However, imprecision in the sample mean and sample standard deviation frequently are greater than expected, while estimates of variability in these parameters are not as well characterized as with population analysis.

A population pharmacokinetic analysis is statistically more robust and designed to generate a full description of the drug's PK behavior in the population in terms of mean parameter estimates with measures of between subject variability and residual variability, including within-subject variability, model misspecification, and measurement error.

Methods of Population Modeling

Currently available methods for population modeling use different algorithms to estimate parameters and their variability within a population as summarized in Table 6.1 (Ette and Williams 2007; Bonate 2006; Kiang et al. 2012). Parametric methods assume a normal Gaussian or log-normal distribution of the parameters and generate means (theta) and account for different levels of variability (between-subject, within-subjects, between occasion, and residual variability estimates). Often routinely collected clinical data distributions are not normally distributed. Nonparametric population models have discrete, not continuous, parameter distributions (Aarons 1992; Jelliffe et al. 1998). Mallet was one of the first to show that the solution for the population model was a discrete probability distribution without any assumptions about the shape and distribution (Mallet 1986). Ideally, the population model would consist of the entire collection of parameter estimates for every patient in that population.

The following summarizes statistical methods applied to population pharmacokinetic and provides an evolution of approaches (FDA 1999).

Naïve Pooling

With this technique all the data are pooled together and analyzed as if they came from a single subject or patient. From the compiled concentration–time profile(s) pharmacokinetic parameters can be estimated. By the nature of the underlying assumptions no information can be obtained on individual variability. A second drawback of this type of analysis is that no information can be obtained in relationships with patient characteristics (e.g., such as age, renal function, etc.). This approach is not recommended but may be helpful to obtain initial estimates before applying a more robust modeling approach.

Standard Two-Stage Approach

The traditional method for population pharmacokinetic analysis used to be the standard two-stage (STS) approach. The first stage of the STS approach is to

Table 6.1 Examples of modeling software based on application. For additional details see Kiang et al. (2012), Sherwin et al. (2012)

Population	Estimation method	Features	Reference
NONMEM	Nonlinear mixed-effects modeling	Sparse and rich data; mixture models, Bayesian, SAEM; parallel processing enabled	Beal et al. (2009)
Pmetrics	Nonparametric Adaptive Grid (NPAG)	Sparse and rich data; R embedded; Iterative 2-stage Bayesian module; semiparametric Monte Carlo simulator; interphase with RightDose software	LAPK (2012)
Monolix	Stochastic approximation expectation maximization (SAEM)	Sparse and rich data; user-friendly GUI	LIXOFT (2012)
Phoenix NMLE	Nonlinear mixed-effects modeling	Sparse and rich data; user-friendly GUI	NMLE P (2012)
WinBUGS	Markov Chain Monte Carlo	Sparse and rich data; user-friendly GUI	WinBUGS (2012)
Individual	*Method*	*Features*	*Reference*
Phoenix WinNonlin	Nonlinear regression	Rich data; compartmental and non-compartmental analysis (NCA); user-friendly GUI	WinNonlin (2012)
ADAPT, S-ADAPT	Weighted least squares, maximum likelihood (ML), generalized least squares (GLS), maximum a posterior Bayesian estimation (MAP)	High-level programs for simulation, data analysis and design of experiments, designed primarily for basic and clinical research modeling and data analysis applications involving pharmacokinetic and pharmacodynamic systems	ADAPT (2012)
Clinical	Method	Features	Reference
Abbottbase	Bayesian algorithm	Not supported anymore	Abbott Laboratories (1991)
MWPharm	Nonlinear regression; Bayesian talgorithm	Patient database and a drug database with over 180 drugs; models include up to 3 compartments for injection, infusion, oral, and intramuscular as inputs. Dialysis and renal replacement (extracorporeal) clearance option; Windows compatible; connectivity to remote database; regular updates	MediWare. Available from http://www.mediware cz index_en html, Proost and Meijer (1992)
BestDose	Population model-based dose calculator	Initial dose prediction of gentamicin, amikacin, and vancomycin .as a part of routine clinical care	Holford (2012)
Doseme	Bayesian algorithm	Bayesian adaptive feedback software for vancomycin and gentamicin dosing	Green (2012)
TCIWorks	Bayesian algorithm	Target concentration intervention software designed for dose optimization for individual patients to be used as a part of routine clinical care, such as aminoglycosides and vancomycin	Kirkpatrick (2012)

RightDose	Nonparametric, multiple-model Bayesian adaptive control	Graphical user interface, customizable, under active development, including linkage with EMRs for automation and web-based service	LAPK (2012)
Simulation	Method	Features	Reference
Berkeley Madonna	Differential equation solver	Fast and convenient, general purpose differential equation solver; multiple integration algorithms	(Berkeley-Madonna 2012)
AcslXtreme	Markov Chain Monte Carlo	Classical pharmacokinetic (PK) physiologically based pharmacokinetic (PBPK) and pharmacodynamic (PD) modeling	(Acslx 2012)
Optimal design	*Method*	*Features*	*Reference*
ADAPT, S-ADAPT	Design module (SAMPLE) provides the ability to calculate D- and C-optimal designs using FIM	Multiple estimation, simulation, and optimal sampling modules; population, STS, and ITS	ADAPT (2012)
WinPOPT and POPT	Optimization of nonlinear mixed effects models	MATLAB based; optimization for multiple response models and responses	WinPOPT (2012)
PFIM	Expression of the Fisher information matrix in nonlinear mixed effects models	R tool allows evaluation and optimization of population designs; user-friendly GUI	PFIM. Available from http://www pfim biostat fr
PopED	Optimal experimental design tool for nonlinear mixed effect models using FIM	Matlab scripts with GUI; Enables post- and preprocessing of optimizations, e.g., adaptive design within the GUI	PopED. Available from http://poped sourceforge net
Trial Simulator	Optimal experimental design tool with common PK, PK/PD, and physiologically based PK models and Monte Carlo simulation options	User-friendly GUI; descriptive statistics, ANOVA, survival analysis, and custom S-PLUS analyses; data export to SAS, S-PLUS, NONMEM, WinNonlin(72); library of prebuilt PK, PK/PD and PBPK models	TrialSimulator. Available from http://www.pharsight com main php
Monte Carlo simulation	Method	Features	Reference
ADAPT, S-ADAPT	Simulation module (SIM)	Multiple estimation, simulation, and optimal sampling modules; population, STS, and ITS	ADAPT (2012)
Crystal ball	Monte Carlo simulation and time-series forecasting	Suite of easy-to-use Microsoft Excel add-in software that helps you analyze the risks and uncertainties associated with your spreadsheet models	Goldman (2002)
MicLab	Object-oriented pharmacokinetic pharmacodynamic modeling language (PML)	Monte Carlo simulations,; graphical display	Medimatics. (2012)

characterize each individual concentration-time relationship by nonlinear least-squares regression using as many data points as possible. Each dataset must have sufficient data to allow the algorithm to fit. Then, in Stage 2a, the population model is defined as the means and standard deviations (SD's) calculated from the individual parameter estimates of the studied patients. Finally, in Stage 2b, relationships between patient characteristics and estimated pharmacokinetics parameters are established by multiple regression techniques. The method generally does well to get mean estimates but systematically overestimates variance. The STS approach limits the number of patients who can be studied because of the elaborate study design.

The many phlebotomies required of these sick patients with frequent poor venous access, in combination with the time and expense associated with obtaining and assaying so many samples, can be a major drawback. This may especially be a problem in specific populations such as neonates, children, and critically ill patients.

Iterative Two-Stage Bayesian Approach

The iterative two-stage Bayesian procedure starts with a set of selected mean parameter estimates and standard deviations, e.g., from a standard two-stage procedure. Next, these initials values are used as "priors" to update each individual estimate by using a maximum a posteriori (MAP) Bayesian algorithm. The new set of individual estimates serves to generate new mean parameter estimates and standard deviations. This iterative process is repeated over and over until a predefined convergence criterion is reached. The advantage of the Iterative two-stage Bayesian approaches (IT2B) procedure is that it can handle sparse data. Furthermore, the distribution of the parameter estimates is better defined than with the STS.

Nonlinear Mixed Effect Modeling

This method of population modeling was first introduced in the late 1970s by Sheiner and Beal (Beal and Sheiner 1982; Sheiner et al. 1977). The acronym NONMEM which stands for Nonlinear Mixed Effect Modeling has become synonym to "population approach," but it also refers to the computer package that initially was made available by this group. To date there are several software packages that provide nonlinear mixed effect modeling capabilities (Kiang et al. 2012; Aarons 1999).

With the population approach all responses from all subjects are analyzed *simultaneously*. In addition, balanced as well as unbalanced data can be simultaneously analyzed with different subjects contributing varying amounts of data. This provides flexibility and allows the analysis of both rich and sparse experimental data.

Table 6.2 Equations for a simple population model as used in NONMEM

General model description	$DV = f(IDV,P)$	DV, dependent variable; *f*, function; IDV, independent variable; *P*, parameters
Structural PK model	$Cp_{ij} = \frac{Dose_i}{V_i} \cdot e^{-\frac{CL_i}{V_i} t_{ij}}$	Cp, plasma concentration; *ij*, *i*th concentration in *j*th subject; *V*, volume; CL, clearance; *t*, time
Variability model (between-subject variability)	$CL_i = CL_{pop} + \eta_{CL,i}$	Fixed effects: CL, clearance; *i*, individual subject; pop, population Random effect; η, eta, between subject variability
Error model(s) (residual variability)	$Cp_{ij} = Cp_{ij} + Cp_{ij} \cdot \varepsilon_{ij}^{prop} + \varepsilon_{ij}^{add}$	ε, epsilon, residual variability (or within subject) prop, proportional; add, additional
Covariate models	$CL_i = CL_{pop} \cdot \left[\frac{WT_i}{70kg}\right]^{0.75} + \eta_{CL,i}$	Allometrically scaled clearance model
	$V_i = V_{pop} \cdot \left[\frac{WT_i}{70kg}\right] + \eta_{V,i}$	WT, weight

A parametric population analysis estimates the central tendency or "typical" (i.e., population mean or median) of the parameters of a user-specified structural model (e.g., clearance and volume), between-subject variability, and residual variability (Table 6.2). Mixed effects models consist of fixed effect and random effect parameters. Typical fixed effect parameters are clearance and volume and the predictive relationships between these parameters and clinical and pharmacogenetic/genomic factors (i.e., covariates). For example, between-subject variability in clearance may be partly predicted by body size (i.e., allometrically scaled weight) and creatinine clearance (Table 6.2). Parameters are assumed to be normally (or log normally) distributed, where η is a random variability with a mean of 0 and a variance of ω^2 (omega squared). Epsilon is the term NONMEM uses to quantify residual variability. As a particular ε cannot be defined (as it is random), its distribution is assumed to be normally distributed, with mean 0, and variance σ^2 (sigma squared) (Broeckman et al. 1994).

Although there is not one universal way to develop population models, the general steps involved are as outlined below (Ette and Williams 2004a, b, 2007; Mould and Upton 2012; Ette et al. 2004; Sherwin et al. 2012).

Step 1: Establishing a database. Data collected in clinical studies are often complex and require extensive cleaning and reformatting. Accurate information is required on patient's demographics, dosing, timing of dose events, sampling of drug concentration and other biomarkers, additional laboratory measurements, and clinical status. Meticulous checking and cleaning of the data before the actual modeling is one of the most important and time consuming first steps and is part of good modeling practice.

Step 2: Base model. The structural PK models can be defined as one-, two-, or three-compartmental models. For orally administered drugs, parameterization typically is

with a first-order (Ka) or zero-order rate constant for absorption, combined with clearance (CL/F), intercompartment clearances (Q/F), and apparent volume of distribution(s) (V/F). Typically data are log transformed (natural logarithm) and modeled using the first-order conditional estimation method with interactions. Between-subject variability (BSV) and between-occasion variability (BOV) are modeled exponentially. Residual error is modeled as additive, a constant coefficient of variation or a combination of the two (Table 6.2).

Step 3: Covariate analysis. Potential covariates are investigated using a stepwise forward addition and backward elimination process. A significance level of 0.05 is typically used in the forward step to allow inclusion of potential covariates. More stringent criteria are used in the backward elimination step (e.g., *P*-values of 0.01 or 0.001) to retain only clinically meaningful covariates in the final model.

Step 4: Model validation. The difference in objective function value (ΔOFV) computed as −2× loglikelihood is used as the statistical criterion for differentiation between hierarchical models. Further assessment and comparison uses the likelihood ratio test and evaluates changes in the objective function value (OFV) between models. Improvement in model fit is determined using the chi-squared distribution with one degree of freedom ($\Delta OFV < 3.84 = P < 0.05$). Models are also compared using the Akaike information criterion (AIC) and Schwarz information criterion (SIC) to discriminate between non-hierarchical models in the selection of a structural model. Goodness-of-fit plots and simulation-based diagnostics are used for model evaluation. Numerical and visual predictive checks are used to assess the predictive performance of the final model. The final model with variability included is then used to simulate outputs (e.g., concentrations) at each sampling time-point and the 95 % confidence interval around the simulated 5th, 50th, and 95th percentiles are overlaid with the same percentiles of observed data to evaluate the predictive power of the model (visual predictive check). The percentages of observations below and above the simulated 5th and 95th percentile can also be calculated for a numerical predictive check. A nonparametric resampling bootstrap analysis to assess model accuracy and stability is performed to verify relative standard errors and confidence intervals of the final population parameter estimates.

Nonparametric Expectation Maximization

The nonparametric expectation maximization algorithm (NPEM) was first described by Schumitzky (1991). It has undergone several iterations and is now as a nonparametric adaptive grid (NPAG) the basis of the Pmetrics package to perform nonparametric and parametric pharmacokinetic–pharmacodynamic population and individual modeling and simulation (Neely et al. 2012; LAPK 2012). Pmetrics can handle sparse data in one- to three-compartment models and which provides joint probability density functions (PDF) of the population parameters and two- and three-dimensional graphical plots of the various marginal densities. The first part is

actually an iterative Bayesian population program in its own right. The function of this program is to provide reasonable estimates of the ranges for the parameters. The program uses these ranges and finds the most likely PDF given these ranges and the data of each patient's dosage regimen and serum levels. The program itself iteratively computes the entire estimated PDF for each parameter. It is similar to the nonparametric maximum likelihood algorithm of Mallet (1986). Pmetrics also provides traditional data, such as the mean, SD, mode, median, skewness and kurtosis, and the covariance and correlation coefficient matrices between the parameters. The program package is well suited for clinical and therapeutic drug monitoring data and can be used in concert with a clinical dose optimization program named "BestDose."

Markov Chain Monte Carlo Population Modeling

Markov Chain Monte Carlo population modeling is similar to the nonparametric maximum likelihood approach and is based on the observation that if each patient's true PK parameter values were known, then the population prior would be represented by a scatter plot of the true parameter values for each individual in the population. Instead of point estimates of parameter values, it is possible to build the population parameter distribution by having a mixture of the individuals' parameter distributions. The mixing coefficient (i.e., weighting factor) would maximize the reduction of variability in the predicted concentration values for the individual. By sampling from the population prior using Monte Carlo methods and calculating concentrations using the PK model, it is possible to predict probability distributions of plasma concentration at any time point (Ette and Williams 2007; Lainez et al. 2011).

Development of a Population Model: An Example

Nonlinear mixed effects modeling (NONMEM) and nonparametric expectation maximization (NPEM) and adaptive grid (NPAG) have been frequently used in the population modeling of antimicrobial agents, and a few of the references are included here (Bulitta et al. 2007; de Hoog et al. 2002; Drusano et al. 2000, 2002; McGee et al. 2009; Peloquin et al. 2008; Preston and Drusano 1996; Vinks et al. 1996, 2003; Yuen et al. 1992). As an example we here describe the analysis of a simple tobramycin dataset analyzed with both NONMEM and the NPAG algorithm to explore some of the program output. Data come from a pharmacokinetic study in a cohort of 470 neonates who received tobramycin therapy as part of standard of care (Table 6.3) (de Hoog et al. 1997, 2002). In this particular study tobramycin was dosed according to gestational age (GA). Premature neonates younger than 28 weeks received 3.5 mg/kg every 24 h; neonates with a GA of 28–36 weeks received 2.5 mg/kg every18 h; and newborns older than 36 weeks GA were dose

Table 6.3 Demographic data if neonates in the tobramycin population modeling study. Adapted from de Hoog et al. (2002)

Variable	Median (range)
Total number of patients	470
Male/female	267/203
Gestational age (weeks)	31.6 (23.7–42.9)
Weight at birth (g)	1,530 (485–5,245)

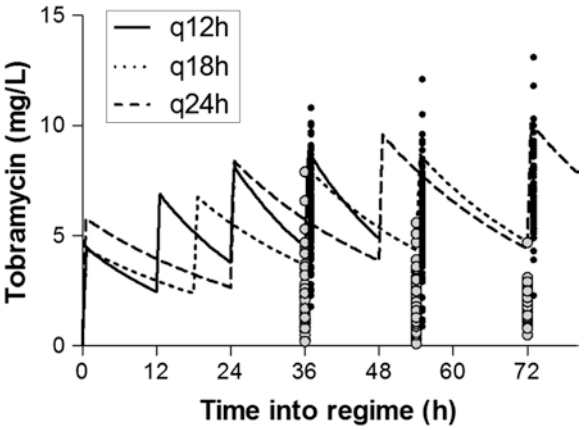

Fig. 6.1 Tobramycin concentration time profiles and observed concentrations (predose trough and postdose peak) after dosing every 12, 36, and 48 h in 470 neonates

2.5 mg/kg every 2 h. Peak and trough concentrations were obtained before and after the fourth dose for each patient as summarized in Fig. 6.1. The population model was parameterized as a one-compartment with first-order elimination rate (Ke), as well as clearance (CL) and volume of distribution (Vd). Covariate analysis revealed weight as a significant covariate predictive of volume of distribution.

A typical summary of graphical output in terms of model predictions and post hoc individual parameter estimates for the parametric (NONMEM) and nonparametric approach (NPAG) are presented in Fig. 6.2. Panels (a) and (b) show the NONMEM output of the frequency distributions of the tobramycin parameter estimates Ke (in h−1) and volume of distribution (expressed as L/Kg bodyweight), respectively. In NONMEM jargon these are called empirical Bayes ("post hoc") estimates (EBE), whereas in nonparametric analysis they are referred to as maximum a posteriori probability (MAP) Bayesian estimates (Panels C and D). As can be expected, the nonparametric algorithm NPAG generates distributions do not represent a normal or Gaussian distribution but rather show a skewed and multimodal pattern. The empirical Bayes estimates generated by NONMEM on the other hand represent normal distributions. An important difference of the NONMEM output is that the probability density functions are confined around the mean. This can be the result of a phenomenon called "shrinkage" (Savic and Karlsson 2009). In addition, differences in the residual error model structure further contribute to differences in output (de Hoog et al. 2002). If the data are relatively uninformative, the EBE distribution will "shrink" toward zero (i.e., the population mean) resulting in a more narrow distribution of the post hoc Bayes values. EBE-based diagnostics should be

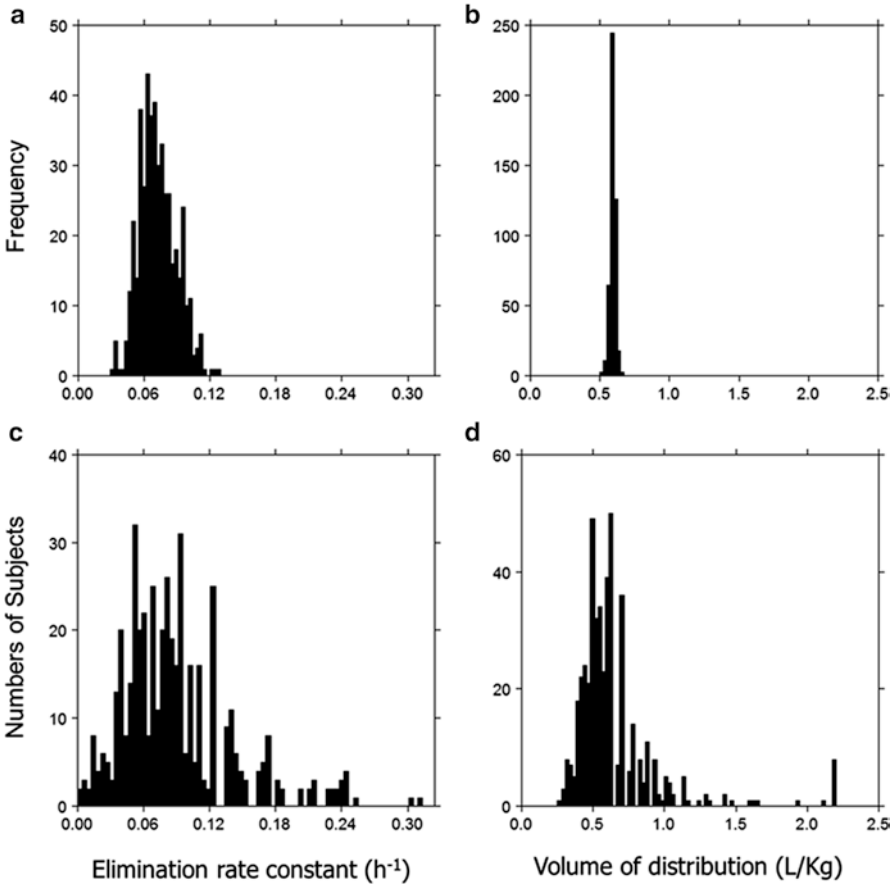

Fig. 6.2 Frequency distributions of tobramycin pharmacokinetic parameter estimates, elimination rate constant (h−1) and volume of distribution (L/Kg) in 470 neonates. Adapted from de Hoog et al. (2002)

interpreted with caution whenever substantial shrinkage exists (usually when greater than 20–30 %). In general, shrinkage indicates that the model is over-parameterized for the data that is available. The first recommendation is to simplify the model if possible. If that doesn't resolve the issue, the second recommendation is to remember that the diagnostic plots may be misleading.

Figure 6.3 (Panels a and b) present the model based and individual Bayesian predicted concentrations obtained with NONMEM, respectively. Panels (c) and (d) present the respective output for the NPAG algorithm in Pmetrics. Differences in the observed versus prediction plots are the result of the fact that Pmetrics uses weight as a covariate in the model predictions (Fig. 6.4c). In addition, the error model in Pmetrics is largely determined by the assay error pattern which results in better aposteriori predictions than NONMEM which estimates larger residual error and shrinkage (Fig. 6.2, Panels d vs. b).

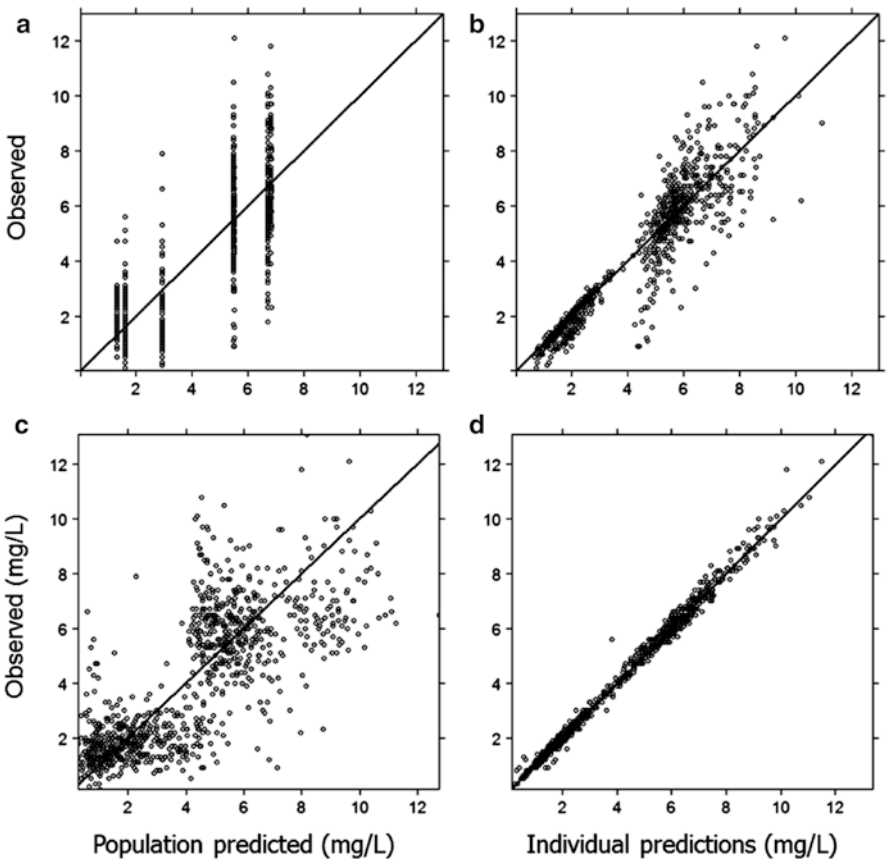

Fig. 6.3 Observed tobramycin concentrations versus population model predicted (panel **a** and **c**) and post hoc Bayesian predictions (Panels **b** and **d**). Shown are peak and trough data for 470 neonates measured after the forth dose. The *solid line* represents the line of identity; coefficients of determination are 0.51 and 0.88 for NONMEM estimates; and 0.71 and 0.99 for NPAG-generated estimates, respectively

Application of Population Models

So far we have discussed population modeling to identify central tendency of PK/PD parameter estimates and quantify between patient variability in the targeted patient population. Next, by using covariate analysis, clinically useful parameters can be included in the model to explain more of the observed variability. For instance when the tobramycin model is parameterized as a clearance model, gestational age was identified as a significant covariate with younger neonates having larger volumes of distribution and slower clearance (Fig. 6.4) (de Hoog et al. 1997).

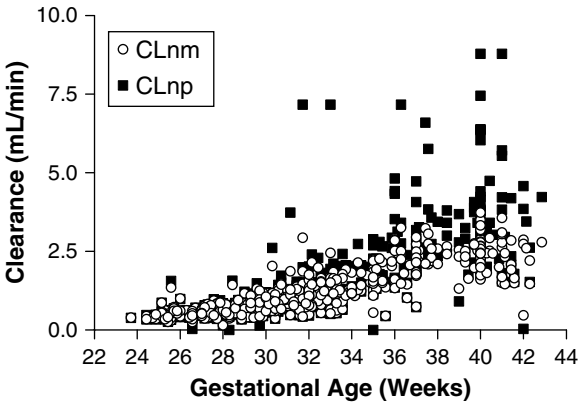

Fig. 6.4 Tobramycin clearance estimates versus gestational age in 470 neonates. *Open squares* represent NONMEM clearance estimates (CLnm); *closed square* represent NPAG estimates (CLnp). Adapted from de Hoog et al. (2002)

Such relationships are important as it captures in this case the effects of growth and development on maturation of drug clearance. In other situations these longitudinal associations will provide insights in the relationship between drug disposition and disease progression (or improvement) over time.

Clinical Trial Simulation

It is interesting to note that the initial impetus to develop population-based methods was spurred in the late 1970s and early 1980s by the clinical need to support the analysis of data obtained from routine drug monitoring to develop individualized dose regimens based on patient-specific covariates (Sheiner et al. 1977). Since then population-based methods have increasingly been implemented particularly in the drug development process (Lalonde et al. 2007). An impressive variety of PK/PD models have already been defined and applied to numerous drugs, and in recent year pharmaceutical companies have embraced the implementation of model-based drug development (MBDD). The concept of quantitative model-based drug development (QMBDD) originated some time ago as the application of quantitative assessments of drug disposition and drug action evolved and was landmarked by the "learn and confirm paradigm" proposed by Lewis Sheiner (1997).

If such modeling is to become even more useful and efficient, a database of accumulated experience (models and results) will be indispensable. Population PK/PD modeling has become quite common and models have been described for many antimicrobial agents and antifungals. Modeling and simulation techniques have enabled us to summarize large amounts of data into discrete distributions that describe the whole dataset and can be used as a priori information to design dosing regimen. Model-based clinical trial design will allow optimization of drug exposure using PK/PD characteristics across the study population. The different steps involved in the design of informative studies are schematically outlined in Table 6.4.

Table 6.4 Schematic representation of modeling and simulation steps to help in the design of informative PK/PD studies. Adapted from Mouksassi et al. (2009)

Step 1	Development of a population PK/PD model using newly generated or prior knowledge
Step 2	Simulation of 'realistic' virtual patients
Step 3	Simulation of the virtual clinical study – Target PK/PD exposure – Dose finding and dosing rules
Step 4	Optimizing of trial design and data analysis method prior to the study

Once the model is established, questions related to dose selection and target exposure levels based on PD indices ($T>$MIC, AUC/MIC, C_{max}/MIC) can be addressed through simulation. In addition, design elements related to number of subjects/patients and sampling frequency can be explored by applying D-optimal design and trial simulation.

Modeling of Target Attainment

Appropriate treatment with antimicrobials involves factors that are difficult to control. Between-patient variability in drug exposure, the distribution of minimum inhibitory concentrations (MIC) of the infecting pathogen, and the patient's clinical status all affect the therapeutic response. Despite these uncertainties, we can estimate the probability of attaining a successful therapeutic outcome in the context of factors that are within our control. A powerful modeling technique to evaluate the adequacy of antimicrobial regimens is Monte Carlo Simulation (MCS). First introduced to the infectious disease community by Drusano and Ambrose, MCS is currently applied for the evaluation of (1) dose–exposure relationships, (2) estimation of susceptibility breakpoints, and (3) pharmacoeconomic studies (Ambrose et al. 1997; Bhavnani 2010; Drusano et al. 2001). For different classes of antimicrobial agents PD indices describing the exposure of unbound free (f) drug in relation to the MIC (fAUC/MIC or f$T>$MIC) have been shown to correlate well with efficacy (Chaps. 1 and 2, Craig) and now contribute significantly to the establishment of MIC breakpoints that differentiate between high and low probabilities of cure (Chap. 3 Mouton) (Ambrose 2006; Mouton et al. 2012). MCS differs from traditional simulation in that the model parameters are treated as stochastic or random variables rather than as fixed point estimates. Between-patient variability in population PK parameter estimates has only recently been recognized as a factor in predicting the outcome in individual patients and establishing breakpoint and targets for clinical susceptibility (Mouton et al. 2005). An important consideration when evaluating target attainment with different dosing regimens is to use PK parameter estimates obtained in the target patient population rather than rely on healthy volunteer data. Initially, Monte Carlo simulation was often performed using PK parameter estimates obtained from healthy volunteers. However, there are several potential problems with this approach. First, it assumes that important conclusions can be

drawn based on data from small numbers of healthy volunteers, as if these parameter estimates and their measures of dispersion are predictive for the entire population. Second, various patient populations may have markedly different PK characteristics; this pertains particularly to groups in which the PK properties of a drug are known to be altered, such as in critically ill patients and patients with cystic fibrosis (CF). When put in perspective through consideration of probability of target attainment (PTA) at the extremes of the probability distribution, parameter estimates obtained from relatively small groups of subjects or patients can produce a reasonable estimate of the clinical susceptibility breakpoint.

Different software packages are available for the modeling of target attainment some of which are listed in Table 6.1. Most programs allow inclusion of the covariance matrix (or correlation matrix) of the parameter estimates used in the simulations, which is essential. If the covariance matrix is not included, variance is overestimated particularly for time above MIC drugs (Mouton et al. 2004). The output consists of a probability distribution, a cumulative probability distribution, and specific confidence intervals over user-defined MIC or longitudinal antimicrobial surveillance data (such as the MYSTIC data base) and PD target ranges. Clinically recommended dosing regimens of the antimicrobial agent under study are simulated in the target patient population with realistic weight and other covariate distributions typically using up to 10,000 subjects with PD targets to give near-maximal bactericidal activity.

Bayesian Forecasting

For individualized therapy at the level of the patient, a next step is to select a desired target goal, such as a desired plasma concentration, area under the concentration curve (AUC) or other measure of antimicrobial exposure, and then define the dosage regimen to best achieve that target goal in that particular patient (Neely and Jelliffe 2008). By measuring serum concentrations as part of therapeutic drug management (i.e., feedback), and estimating the most likely set of individual parameters estimates (individualized model) given the population priors (population model) of how the drug is behaving in that patient, one can further optimize the dosing regimen. This process is called Bayesian forecasting or Bayesian adaptive control (Jelliffe et al. 1993, 1998). Bayesian analysis is a particular useful approach in information-sparse environments as encountered in clinical practice and as part of prospective clinical trials. The method of Bayesian forecasting is derived from Bayes' theorem and is based on the concept that prior PK knowledge of a drug, in the form of a population model, can be updated with individual patient data, such as drug concentrations (Jelliffe et al. 1998, 2000, 2011; Jelliffe 2000). The idea is to make an individualized model of the behavior of the drug in a particular patient to see how the drug will be or has been handled and to obtain the necessary information to make rational dose adjustments so as to best achieve the selected target goal(s).

Table 6.5 Flow scheme of Bayesian forecasting (Jelliffe et al. 1998)

Bayes Theorem	Prior probability	New information	Consider prior and new information	Posterior probability	Therapeutic Goals	Control
PK/PD model-based guidance	Population model	Drug concentration	Objective function	Individual model	Look at patient. Set your goals and targets	Calculate new dosing regimen

Table 6.5 summarizes the stepwise process for the model-based optimization process. Drug dosage optimization requires (a) the population model, defined as summary of support points (Neely et al. 2012) or as mean values, standard deviations, covariates, and information on the statistical distribution necessary to select the initial dosing regimen for that particular patient based on chosen goals; or (b) measurement of a performance index related to the therapeutic goal, generally one or more plasma concentrations or effects as feedback information to update the system; (c) availability of reliable software for an adaptive control strategy (maximum a posteriori probability [MAP] Bayesian fitting) and calculation of the subsequent optimal dosage regimen. Recently a nonparametric multiple-model Bayesian adaptive controller became operational to achieve and maintain selected therapeutic goals with optimal precision (LAPK 2012; Macdonald et al. 2008; Jelliffe et al. 1994).

For several classes of antibiotics, clinically useful models have been developed that can be used for dose individualization, even when drug concentration measurements are not available or considered necessary. Application of these models is particularly useful in critically ill patients, patients with organ failure, and when placed on renal replacement therapy.

PK/PD Model-Based Antimicrobial Therapy

In clinical practice, the emphasis of measuring antibiotic drug concentrations has been mostly on narrow therapeutic index drugs such as aminoglycosides and vancomycin. Many clinical laboratories offer some form of aminoglycoside and vancomycin TDM. However, pharmacokinetic interpretation is frequently not an integral part of services provided. As a result dose adjustments frequently are made on an "ad hoc" basis relying on numbers of one or more concentration measurements and solely considering a "therapeutic range." This approach is called "reactionary" TDM where a standard dose is administrated and the concentration is checked to verify whether it is in the "therapeutic range." Interventions typically are toxicity driven. If the level is too high (i.e., above the therapeutic range), the dose will be lowered and the level will be checked again. If the level is too low, the dose may be increased and the concentration measurements repeated until "therapeutic." If the first measurement is within the therapeutic range, things are considered okay and no

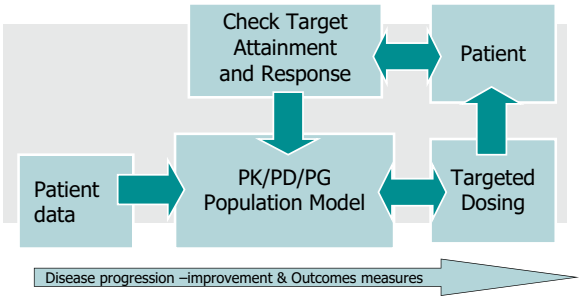

Fig. 6.5 Schematic representation of the target-controlled model-based individualized dosing strategy. A clinical pharmacokinetics program with a patient-specific population model describing absorption, distribution, and elimination of the antibiotic in relation to patient-specific parameters is used. Patient data and desired target concentrations are entered into the system. Next, a model-based loading dose and maintenance regimen required to optimally achieve the target concentrations is determined. This regimen is administered to the patient and subsequent concentration measurement(s) are used as feedback to check target attainment and update the model and/or design a new dosing regimen, if required. *PK* pharmacokinetics, *PD* pharmacodynamics, *PG* pharmacogenetics

further action is taken. Such reactionary form of TDM does not take into consideration the full concentration–time profile as well as specific pharmacodynamic targets and will not lead to efficient use of resources and good outcomes. For aminoglycoside monitoring, many studies have documented that TDM guidance improves overall use of resources resulting in fewer inappropriately drawn samples, more peak and trough levels in the target range, fewer dose adjustments and reduction of the incidence of nephrotoxicity (Ensom et al. 1998; Touw et al. 2005). Ideally, a process of therapeutic drug management is adopted that combines the measurement of drug levels with the application of pharmacokinetic and pharmacodynamic principles. A few prospective studies using population models and Bayesian feedback have shown the superiority of model-based PK/PD guidance, both in terms of cost effectiveness and better clinical outcomes including better survival (van Lent-Evers et al. 1999; Leon-Djian et al. 2011).

The PK/PD model-based dosing strategy comprises two important steps. First, explicit therapeutic goals for each patient should be defined. Second, a strategy to achieve these goals with the greatest possible precision should be chosen. Figure 6.5 shows an overview of the optimization process. Drug dosage optimization requires (1) population pharmacokinetic parameters, including mean values, standard deviations, covariances, and information on the statistical distribution to select the initial dosing regimen for that particular patient; (2) measurement of a performance index related to the therapeutic goal, generally one or more plasma concentrations as feedback information to update the system; and (3) availability of reliable software for adaptive control strategy (MAP Bayesian fitting) and calculation of the subsequent optimal dosage regimen. Clinical examples of the application of this population model-based dose optimization for antimicrobial dosing of antimicrobials (e.g., tobramycin and ceftazidime) in critically ill patients receiving renal replacement

therapy have been described (Vinks 2002; Reetze-Bonorden et al. 1993; van Dalen and Vree 1990).

In a similar fashion, population model-based dosing was implemented in a group of patients admitted to the hospital with proven or suspected Gram-negative infection as part of a prospective cost-effectiveness study (van Lent-Evers et al. 1999). In the study clinical outcomes after standard of care and implementation of PK/PD model-based intervention were compared. The model-based intervention differed in that antibiotic concentrations were measured immediately following the first dose (at $t = 1$ h and 8–12 h) and reported back with a recommendation before the next dose. Concentration measurements were used as feedback to the model and provided individualized dosing regimen based on patients' clinical condition and infection-specific targets. This approach proved more effective less costly and reduced mortality, hospital stay, and adverse events in the patients admitted with Gram-negative infections. In addition, it greatly improved communication between physician, laboratory staff, and other care providers.

However, regardless of what PK/PD dose individualization techniques are used, all are superior to a simple-minded comparison of a result to a therapeutic range. Simply reporting results as below, within, or above a published range, are usually uninformative, not cost saving, and can lead to inappropriate actions.

Conclusions

Great progress has been made in the field of population PK/PD modeling. With the available clinical software, population models can now assist in developing individualized antibiotic dosing regimens. Also during the past decade, the development of useful in vitro and animal models has expanded our knowledge of the pharmacodynamics of antimicrobial agents. The next step is to integrate our knowledge of pharmacokinetic behavior with pharmacological and clinical effects. Application of such integrated mathematical models has demonstrated to correlate with microbiological and/or clinical outcomes. In addition, the design of dosing regimens whether in early drug development or to individually optimize treatment in clinic will greatly benefit from such PK/PD population models. This will eventually provide us with a better rationale for the proper selection of optimal dose, type, and duration of administration of anti-infective therapy in different patient populations.

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Chapter 7
Suppressing Resistance Development

Vincent H. Tam

Abstract Earlier studies of pharmacokinetics (PK) and pharmacodynamics (PD) have focused on optimizing antimicrobial agent exposures to (1) improve clinical outcomes and/or (2) reduce the likelihood of drug-induced toxicity. As the discipline matures, some studies have examined the relationship between antimicrobial agent exposures and the development of resistance. Using prior PK/PD concepts as foundation, various experimental designs (e.g., in vitro, in vivo, computer model based) and mathematical heuristics (e.g., surrogate PK/PD indices, differential equations, stochastic simulations) have been put forward. While there is no consensus on the best method used, there is clear experimental evidence that dosing regimen (dose, dosing frequency, duration of drug administration, duration of treatment) design could influence the development of resistance under certain circumstances. Rational dosing regimen design is therefore increasingly recognized as part of a comprehensive solution, in conjunction with infection control and antimicrobial stewardship, to suppress (delay) the emergence of resistance in the clinical setting.

Keywords Counter-selection • Resistance emergence • Drug development • Modeling • Treatment • Dosing regimen design

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Background

Emphasis of Prior Efforts

The concept of pharmacokinetics (PK) and pharmacodynamics (PD) had a major impact on how dosing regimens of antimicrobial agents are designed in the past decades, both in the drug development and the clinical settings. Earlier studies focused on optimizing drug exposures to improve outcomes (clinical cure and/or microbiologic eradication), as exemplified by the two most often-cited milestone papers (Forrest et al. 1993; Preston et al. 1998). Following these examples, numerous investigations have been undertaken in a variety of clinical and experimental settings. An excellent review has recently been published discussing major findings in these studies (Ambrose et al. 2007).

Almost at the same time, there was much interest in understanding the relationship between drug exposures and drug-induced toxicity. Clinical investigations in this area provided important insights to ways to improve the benefit to risk ratio of aminoglycosides (Moore et al. 1987; Verpooten et al. 1989), which led to studies aiming to confirm these findings (Kashuba et al. 1999; Rybak et al. 1999). These key studies have resulted in wild spread adoption of once-daily (extended interval) dosing of aminoglycosides across the nation (Chuck et al. 2000).

Resistance on the Rise

With the advent of antimicrobial resistance on the rise, many first-line agents have been rendered ineffective (Flournoy et al. 2000; Neuhauser et al. 2003; Gaynes and Edwards 2005). The situation is dire given a lack of new agents in the developmental pipeline (Spellberg et al. 2004; Spellberg 2008). Grave concerns have been raised about these rising resistance trends and the urgent need for effective strategies to combat them (Talbot et al. 2006; Arias and Murray 2009). Using some of the previous PK/PD concepts as foundation, the feasibility of optimizing an antimicrobial agent exposure to suppress resistance development has been reviewed (Drusano 2003). The central theme is to prolong the clinical utility of existing agents by using them more appropriately. Over the years, various experimental setups and notions have been put forward. Although no consensus has been reached conclusively among experts in the field, there are unambiguous experimental data demonstrating the influence of dosing exposure on resistance development. Although a similar analogy in drug effect and microbial population dynamics can be found in other infectious agents (e.g., HIV, fungi, parasites), this chapter will focus on work and advances relating to bacterial resistance.

Factors Influencing the Development of Resistance

Mechanism of Resistance

While a comprehensive review of all bacterial resistance mechanisms is beyond the scope of this chapter, some general comments can be made with the majority of relevant studies. Most of the studies published looked at vertical resistance transfer, commonly conferred by chromosomal mutation(s) and/or transcriptional changes. In examples where optimal drug exposures had an influence of resistance development, the extent of susceptibility reduction in resistant mutants was usually not drastic (<4–16× increase in MIC from baseline). On the other hand, horizontal spread of resistance often involves acquisition of genetic elements coding for drug resistance (e.g., transfer of extended-spectrum beta-lactamases). The subsequent change in MIC in the recipient strain is expected to be much more significant. Thus the likelihood of suppressing this type of resistance by optimal dosing regimen selection is not high.

Natural Resistant Mutants

When a bacterial population is greater than the inverse of the frequency of natural mutation, it is likely that natural resistant mutants would be present at baseline. Although present in a very small proportion, these pre-existing mutants are expected to have survival benefits (compared to their wild-type counterparts) under selective antimicrobial pressure from a suboptimal drug exposure. As a result, the composition of the bacterial population will be altered over time, resulting in a more resistant phenotype. Many PK/PD investigations of resistance suppression thus used a higher than standard starting inoculum (e.g., $>10^7$ CFU/ml). Alternatively, bacteria with impaired mismatch repair mechanisms (e.g., *mutS* or *mutL*) have also been used. These bacteria have a significantly higher baseline mutational frequency (hypermutable), thus pre-existing resistant mutants could be present even at a lower density (Oliver et al. 2004).

Immune Function

Intuitively, an intact immune system would enhance bacterial killing, offering additional effect to that provided by the antimicrobial agents. However, the relative contribution of the immune function to the overall killing is difficult to quantify. Consequently, it may be desirable to first examine PK/PD relationships in a neutropenic (in vitro or in vivo) environment, where a direct relationship between drug exposure and bacterial behavior (unambiguous pharmacodynamic response) could be delineated. The presence of a functional immune system could be considered subsequently as an add-on bonus in marginal circumstances.

Drug Exposure

In appropriately designed experiments, the emergence of resistance has been shown to be linked to the magnitude of drug exposure. However, the relationship was biphasic and took the shape of an inverted-U (Zinner et al. 2003; Tam et al. 2007a). As discussed above, resistant subpopulations represent a very small proportion of the bacterial population at baseline. At a low dosing intensity range, resistant populations were first preferentially selected out as they had a more favorable competitive advantage over their wild-type counterparts. With further increase in drug dosing intensity, more and more resistant subpopulations could be inhibited until all populations were suppressed. Therefore there is a drug exposure target which could be used to suppress resistance development. For fluoroquinolones, this target exposure required to suppress resistance is higher than those reported for favorable clinical outcomes (Ambrose et al. 2003). This could be interpreted as a higher demand of the pharmacologic effect. The difference could also be attributed (at least partially) to the effect of the immune function, as most clinical studies involved immunocompetent hosts.

Time of Drug Exposure

The duration of treatment for various infections is often a hot topic of debate in the clinical setting; as very few clinical investigations focus on this aspect of dosing regimen design. Available clinical observations suggest that while some antimicrobial therapy is good, longer is not always better (Chastre et al. 2003; Shorr et al. 2006). The nonlinear time dependence of resistance development was further exemplified by our experimental data (Tam et al. 2007b). In an in vitro infection model, the development of quinolone resistance in *Staphylococcus aureus* was related to both the daily drug exposure (expressed as AUC/MIC) and the duration of therapy. As shown previously, the influence of time on resistance development was biphasic: increasing resistance suppression up to a certain time threshold, upon which the initial benefit would be nullified/offset by further increase in the duration of treatment.

This observation added further complexity to the pharmacodynamic relationship and provided insights from an additional dimension to previous studies: there must be sufficient time (and repeated dosing) in a study for resistance emergence to be apparent. Typical PK/PD studies lasting for 24 h would thus have a limited ability to detect resistance emergence, despite using the most conducive selective pressure. The ideal duration of investigation is not known, but should probably take into consideration the growth rate of the pathogens under investigation. Resistance development has been demonstrated in studies lasting from 2 days (Tam et al. 2005a) to more than 2 weeks (Drusano et al. 2008). Furthermore, the use of selective media (often supplemented with a low concentration of the drug under investigation) would be very helpful to track the composition of a bacterial population over time.

Regardless of the trend of the total bacterial burden, selective amplification of a subpopulation with reduced susceptibility could be used as a sensitive indicator of early resistance development. Taken these data collectively, a rational treatment strategy to suppress resistance is consistent with Ehrlich's statement in his 1913 presentation: "Frapper fort et frapper vite" (hit hard and hit fast) (Tillotson 2001).

Complexity of Dosing Regimen Design

Too Complex for Comprehensive Testing of All Possibilities

There are several modifiable factors in the design of a dosing regimen: (1) the dose to be given; (2) dosing frequency; (3) duration of administration (for intravenous infusion); and (4) duration of therapy. Ideally, testing of all possible regimens should be undertaken to identify the optimal regimen. However, in view of the numerous possibilities involved, a comprehensive testing of all possible regimens is often not practical and financially prohibitive in preclinical and clinical studies (see Appendix 1). Consequently, a systematic approach must be used to guide testing in a rational manner (Fig. 7.1).

Modeling Goal

The purpose of PK/PD modeling is to objectively characterize the relationship between drug exposures and outcomes. Once a relationship is developed, it could

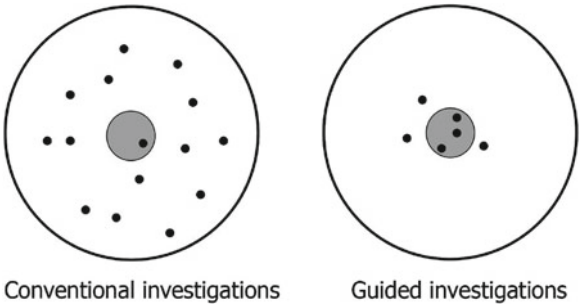

Fig. 7.1 Conventional (*left*) and PK/PD (*right*) approach to selection of antimicrobial agent dosing regimens. In view of the inordinately large number of dosing regimens (all possibilities within the *outer circle*) that would have to be comprehensively tested before the most effective dosing regimens (within the *shaded area*) are identified, conventional testing is often empiric and poorly guided (*dots*, *left*). The PK/PD approach aims to significantly improve the dosing regimen selection process by offering guidance to highly targeted testing (*dots*, *right*), thus reducing unnecessary testing of ineffective regimens and avoiding early abandoning of potentially successful regimens

be used to predict outcomes under a variety of conditions in a comprehensive and efficient fashion. Since the initial observations can be characterized by more than one reasonable way, the predicting ability of a model (beyond the initial conditions and time frame used in development) is a critical component of model development. Its value could not be further emphasized and should be part of the routine model evaluation.

In the context of resistance suppression, the impact of various factors in dosing regimen design mentioned above should be examined in the model development phase. In a preclinical setting, there may be more flexibility to control for several confounding variables and to simplify a complex clinical situation to be examined in a systematic fashion. It is especially so when certain pharmacokinetic parameters are almost always highly correlated (e.g., C_{max} and AUC) in patients. Depending on the drug and pathogen of interest, it may be sometimes more beneficial to focus on just one or two factors, using a dose escalation and/or fractionation design (Louie et al. 2001; Tam et al. 2005d).

Different Approaches to Delineate the Drug–Pathogen Relationship

Fundamental Selection Concept

The prevailing conceptual framework in resistance suppression is depicted in Fig. 7.2. A heterogeneous bacterial population consists of a majority susceptible population and a minority resistant population. In the absence of a selective pressure, the total bacterial population increases overtime, but the relative proportion of the resistant population to the total population remains relatively constant (no resistance emergence). When the heterogeneous bacterial population is subjected to an

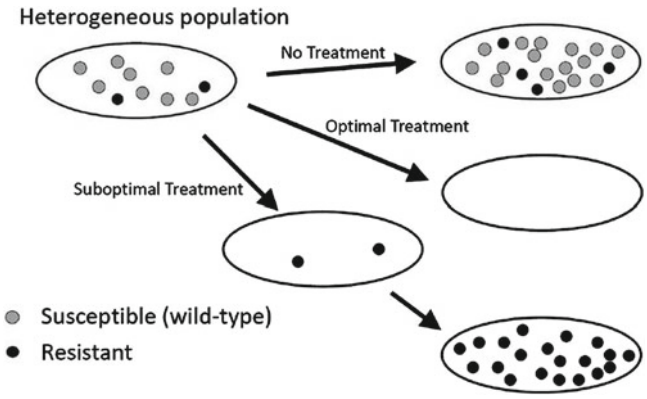

Fig. 7.2 Concept framework of resistance selection under drug selection pressures

optimal drug exposure, all populations (susceptible and resistant) are suppressed and resistance would not emerge. However, in the presence of a suboptimal drug exposure, only the susceptible population is inhibited. The minority resistant population would proliferate without any opposition. The selective amplification of the resistant population over time would ultimately lead to the emergence of resistance.

Standard Methods Not Informative

Minimum inhibitory concentration (MIC) is the most established and widely used method in drug susceptibility testing. The experimental setup is well documented and highly standardized by the CLSI (Clinical and Laboratory Standards Institute 2007). While very commonly used clinically to guide clinicians in the selection of antimicrobial agents, MIC alone may not be very informative for a robust PK/PD analysis to predict the likelihood of resistance emergence (Mueller et al. 2004). At a minimum, some measures of a drug exposure are also necessary: a larger drug exposure might be necessary for a pathogen with a lower susceptibility. Some insights could be gleaned from conventional PK/PD concepts, but for more precise predictions, more sophisticated approaches must be adopted to address the factors mentioned earlier in section "Factors Influencing the Development of Resistance."

PK/PD Surrogate Indices

Inspired by conventional PK/PD concepts focusing on clinical outcomes and toxicity, surrogate PK/PD indices are used in many early studies. These indices reflect a fundamental principle in antimicrobial therapy: the outcome is linked to neither drug exposure nor pathogen susceptibility alone, but rather a complex interplay of both factors. Among these indices, the most widely used are AUC/MIC, C_{max}/MIC, and %T > MIC (Fig. 7.3). Over the years, additional indices (e.g., C_{min}/MIC, AUC/MPC, $\%T_{in}MSW$) have been proposed to better characterize data under certain experimental setting (see below). The choice of these indices is often empiric and additional useful indices could be expected in the future.

In light of the many limitations with standard MIC testing to predict resistance development, a novel concept of mutant prevention concentration was proposed (Blondeau et al. 2001). The basic notion is that high-level resistance is the result of a series of independent events, each associated with its mutational frequency. If the first-step mutant can be inhibited by an elevated drug concentration, then subsequent mutations leading to high-level resistance would not occur. Therefore the mutant prevention concentration can be thought of as the MIC of the first-step mutant. The determination of mutant prevention concentration requires different testing conditions and consequently could lead to additional surrogate PK/PD indices such as AUC/MPC, C_{max}/MPC, and %T > MPC (Fig. 7.3).

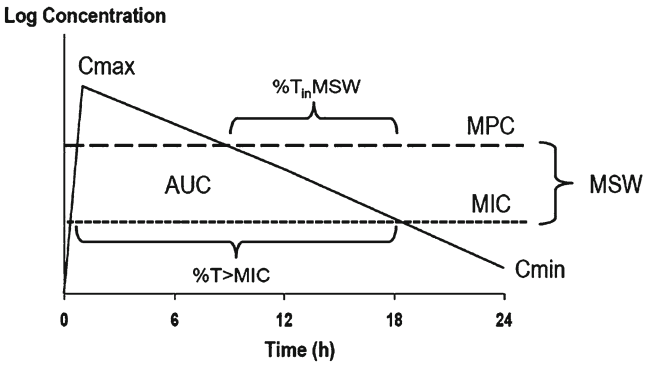

Fig. 7.3 Common PK/PD surrogate indices

While conceptually appealing, the implementation of the MPC concept to clinical practice is not as straight forward. This concept was further developed into a mutation selection window, a concentration range between the MIC and MPC (Zhao and Drlica 2001, 2002) (Fig. 7.3). If a concentration–time profile (resulting from a dosing regimen) has a long residence time within the mutation selection window in each dosing interval (%T_{in}MSW), enrichment of resistant population is expected to be more likely. However, identical %T_{in}MSW could be the result of different dosing regimens. It is currently not known if a universal threshold %T_{in}MSW exists for all classes of antimicrobial agents and pathogens.

There are undoubtedly advantages of using surrogate PK/PD indices. They are intuitive and relatively easy to explain. The interplay between drug exposure and pathogen are exemplified by a combination of conventional terms in pharmacokinetics (e.g., AUC, C_{max}, C_{min}) and microbiology (e.g., MIC). Once a desired effect (e.g., bacterial stasis or 2-log reduction in bacterial burden) is identified, the magnitude of PK/PD exposure can be used as a target in stochastic pharmacokinetic forecasting. The probability of various dosing regimens in attaining these targets can be based on prior knowledge of pharmacokinetic variability in a patient population and relevant susceptibility distribution (Tam et al. 2003).

Dynamic Approach

Given the popularity in the use of PK/PD surrogate indices, it should be pointed out that they are associated with some limitations. By far, most analyses using PK/PD surrogate indices are cross-sectional in nature. Only experimental data observed at one time point was used in the discrimination of various indices (model fits). The approach may be problematic as highlighted previously (Tam et al. 2005c). Since time is a critical factor in the development of resistance, it matters when the experimental observations are made. Different conclusions may be drawn from the same drug-pathogen-dosing combination experiment at different times (Table 7.1).

Table 7.1 Time dependency of pharmacodynamic thresholds to achieve identical endpoints in bacterial burden reduction

		Pharmacodynamic exposure (C_{min}/MIC) to achieve			
Time	R^2	Stasis	1-log drop	2-log drop	3-log drop
24 h	0.993	0.3	0.5	0.7	1.2
48 h	0.999	0.4	0.7	1.0	1.8
72 h	0.979	0.4	0.9	1.6	2.8
96 h	0.985	0.8	1.5	2.3	3.5
120 h	0.993	1.2	1.8	2.6	3.5

Secondary data analysis from Tam et al. (2005b). Targets determined by an inhibitory sigmoid E_{max} model. Despite a satisfactory model fit at each time point, the minority resistant subpopulation plays a more prominent role in the overall bacterial behavior as time progresses. A higher drug exposure is required to achieve an identical effect

Furthermore, it may be difficult to predict bacterial behavior beyond the original time frame of the experiment with so limited input information. More intermediate data must be collected longitudinally.

The dynamic approach would offer a better perspective of the microbial dynamics under an antimicrobial selective pressure over time. As in the case of PK/PD surrogate indices, there is also no standardized dynamic approach to characterize the pharmacodynamic relationship. The fundamental concept of practically all dynamic approaches is to characterize the rate of change of bacteria over time, as proposed in a pioneer study (Zhi et al. 1988). Observations of bacterial burden from time-kill studies with static drug concentrations (Yano et al. 1998; Regoes et al. 2004; Tam et al. 2005c; Nielsen et al. 2007) and more sophisticated experimental setup with fluctuating drug concentrations have both been modeled (Mouton et al. 1997; Gumbo et al. 2004; Meagher et al. 2004; Campion et al. 2005; Tam et al. 2005a, 2007b) using this approach.

A wide variety of mathematical model structures have been proposed. These diverse models could be categorically divided into two major subgroups: a multi-population approach and an adaptation approach. In the multi-population approach, more than one differential equation was used simultaneously; each equation was intended to characterize the behavior of one bacterial subpopulation (often with different drug susceptibility) (Mouton et al. 1997; Gumbo et al. 2004; Meagher et al. 2004; Campion et al. 2005; Tam et al. 2005a, 2007b; Chung et al. 2006). The regrowth phenomenon and resistance emergence were characterized by the relative proportion of different subpopulations in the total population. On the other hand, only one differential equation was often used in adaptation models to characterize the rate of change of the total bacterial population. Resistance development was attributed to adaptation over time, which could be expressed mathematically in more than one way (Schuck et al. 2005; Tam et al. 2005c; Nielsen et al. 2007). Although these modeling approaches are widely diverse in concepts, overall no one approach can be deemed as superior in capturing the input observations. However, some investigations incorporated an additional validation phase beyond the conditions of the input data. These models could thus be considered as more developed models.

Available Evidence

In-Vitro Data

Numerous in vitro studies have been published demonstrating the impact of dosing exposure on the likelihood of resistance emergence. A comprehensive review of all the investigations is beyond the scope of this chapter, thus only a selected few could be highlighted.

Quinolone resistance has received the majority of attention in the past decades. A wild variety of pathogens such as *Streptococcus pneumoniae* (Allen et al. 2003; Zinner et al. 2003; Jumbe et al. 2006), *Staphylococcus aureus* (Allen et al. 2004; Campion et al. 2005; Chung et al. 2006; Tam et al. 2007b; Firsov et al. 2008), *Pseudomonas aeruginosa* (Blaser et al. 1987; Tam et al. 2005a; Nikolaou et al. 2007), *Bacillus anthracis* (Deziel et al. 2005), *Yersinia pestis* (Louie et al. 2007) and *Mycobacterium tuberculosis* (Gumbo et al. 2004, 2005) have been examined. Invariably, chromosomal mutation based resistance (in quinolone resistance-determining regions of genes encoding for topoisomerases) developed under a sub-optimal dosing exposure in almost all the studies.

There are also similar but fewer examples for beta-lactams (Mouton et al. 1997; Tam et al. 2005b; Henrichfreise et al. 2007), glycopeptides (Lubenko et al. 2008) and aminoglycosides (Louie et al. 2007; Tam et al. 2008). In a wide range of in vitro experimental settings, resistance development was observed and linked as a function of drug dosing intensity.

In Vivo Data

In contrast, there are far fewer in vivo studies published examining the emergence of resistance relating to drug exposures. Many animal PK/PD studies were not designed optimally to address resistance development; issues such as the inoculum size, duration of therapy, and immune function have been discussed above. In addition, it is believed there are multiple technical constraints in animal systems, hindering investigations in various aspects. For example, prolonged survival of the animals is a prerequisite for resistance development to be apparent; excessive premature animal mortality will thus make the true benefits of a dosing exposure obscure. Drug metabolism and/or elimination in animals may be much more rapid than in humans, therefore, extra precautions must be taken to simulate humanized drug exposures in animals. Intact (or residual) immune function in animals may also make the data more difficult to interpret. In spite of that, two studies deserve to be highlighted.

In a murine thigh infection model of *P. aeruginosa*, the likelihood of resistance emergence was demonstrated with different dosing exposures of levofloxacin (Jumbe et al. 2003). Immunocompetent mice were infected with a high inoculum of

wild-type *P. aeruginosa*; treatment with a suboptimal exposure resulted in regrowth and the emergence of resistance. On the other hand, an optimal dosing exposure led to sustained suppression of the bacterial burden. This study was the first proof-of-concept study delineating the importance of dose selection to minimize in vivo resistance, providing important evidence supportive of the in vivo relevance of other in vitro observations.

In addition, in a rabbit pneumonia model, enrichment of high-level resistant *Streptococcus pneumoniae* was demonstrated when a first step low-level resistant isolate was exposed to a humanized moxifloxacin dosing exposure (Etienne et al. 2004). Significant reduction in bacterial burden and no elevation in MIC was detected in isolates recovered when the duration of drug concentration within the mutation selection window was minimal.

Clinical Studies

There has not been many clinical study published to date directly addressing the impact of dosing exposure on resistance development. It should be recognized that most clinical studies are designed with respect to a specific disease state (e.g., ventilator-associated pneumonia), rather than a specific pathogen (e.g., staphylococcal diseases). Therefore, most clinical data in this regard were derived by comparing/combining different clinical investigations or as sub-group analyses, focusing on a pathogen (or a pathogen class) of interest. In some studies, microbiologic success/eradication had been used as a surrogate marker of resistance suppression. This correlation was based on the concept that "a dead bug cannot mutate." However, the interpretation of microbiologic failure/persistence as resistance development was more problematic, since susceptibility retesting and/or molecular investigations was not routinely performed to confirm the development of resistance.

In one of the often-cited studies, four prospective clinical investigations were pooled for a combined analysis (Thomas et al. 1998). These studies involved different antimicrobial agents (from structurally distinct classes) used for nosocomial lower respiratory tract infections. Despite the antimicrobial agents investigated were associated with different concentration-killing profiles, a significant relationship between drug exposure and resistance development was reported using a universal pharmacodynamic surrogate index across all microorganisms involved. The overreaching validity of this study has not yet been verified.

Some convincing data could be derived from a clinical study in which patients with nosocomial pneumonia were treated with a prolonged infusion of meropenem. Full details of the study have not been published (personal communication. Drusano GL). In this study, nine patients assigned to the meropenem (2,000 mg every 8 h) plus tobramycin (5 mg/kg every 24 h) regimen had *P. aeruginosa* as a baseline pathogen. These patients were given meropenem as a 3-h prolonged infusion, a PK/PD optimized dosing regimen. Only one patient (11.1 %) was found to

have emergence of resistance by day 7. Compared to historic data in which *P. aeruginosa* resistance developed in a significant proportion (approximately 50 %) of patient with nosocomial pneumonia (Calandra et al. 1986; Fink et al. 1994), the PK/PD optimized dosing regimen of meropenem was promising to suppress resistance development.

Currently Unanswered Questions

Inoculum Effect

Since pre-existing resistant mutants are often necessary in investigations examining resistance development over time, it is important that a high inoculum (significantly more than that used in susceptibility testing) is used at baseline. Multiple investigations have reported that the bactericidal activity could be dramatically reduced when an antimicrobial agent is exposed to a high density of bacteria (Eng et al. 1984; Mizunaga et al. 2005; Tam et al. 2009). However, this observation has not been routinely incorporated in the modeling process to explain our experimental observations. Recently there were two pioneer papers attempting to address this issue (Bulitta et al. 2009; Udekwu et al. 2009), and more work are necessary in this direction.

Mutation During Exposure

Practically all models examining resistance development so far are functionally selection models; they are based on the fundamental assumption that resistant mutants are present at baseline. Under a selection pressure, these mutants have a survival advantage and their relative presence in a population is enriched over time. There are limited experimental data to suggest that resistance could also emerge from a bacterial population of low density. Since the presence of pre-existing resistant mutant is unlikely, resistance is believed to arise due to (transient) transcriptional changes and/or mutation(s) after the bacteria have been exposed to the antimicrobial agents. Consequently, a different conceptual framework to explain the experimental observations would be necessary.

Collateral Damage

There have been concerns raised about resistance development as an ecologic consequence of heavy antibiotic consumption; the analogy cited that of an innocent

bystander (Paterson 2004). There are very limited experimental data that provided insights on how this question can be studied in a more systematic fashion (Goessens et al. 2007). Undoubtedly, more studies are needed from the ecologic perspective of the institution.

Biofitness of Resistant Mutants

In most PK/PD analyses, resistant mutants emerged are commonly assumed to have growth rates and characteristics similar to their wild-type counterparts. The intricate relationship among the molecular mechanism of drug resistance, biofitness cost, and virulence should be investigated further and integrated into PK/PD models.

Probability of Target Attainment from Pharmacokinetic Variability

Despite dynamic models offer benefits in tracking the microbial dynamics over time, they are computationally more demanding. The drug concentration–effect relationship is often described by multiple model parameters, and comparison among several dosing regimens may not be straight forward if more than one parameter estimate is different [e.g., a high maximal kill rate and low C_{50} (drug concentration to achieve 50 % of maximal kill rate) versus a low maximal kill rate and high C_{50}]. It would be desirable if key pertinent information in a complex system were captured and integrated into a composite assessment score for the purpose of stochastic forecasting and ranking different antimicrobial agent dosing regimens (Nikolaou et al. 2007; Tam et al. 2008).

Conclusion

Substantial experimental evidence has amassed over the years demonstrating the impact of dosing exposure on the likelihood of resistance development. Multiple viable approaches have been used to describe/predict these phenomena in a wide range of setting. Limited clinical evidence available appears to support these concepts, but more data are needed from appropriately designed clinical investigations.

Appendix 1: Complexity in Dosing Regimen Design

To evaluate:

Seven daily doses (e.g., 250 mg, 500 mg, 1 g, 2 g, 4 g, 6 g and 8 g).
Four dosing frequencies (e.g., every 6, 8, 12 or 24 h).
Five dosing infusion times (e.g., 0.5, 2, 4, 6 h and continuously over 24 h).
Five duration of treatment (e.g., 3, 5, 7, 10, 14 days).

A total of 700 ($7 \times 4 \times 5 \times 5$) regimens would have to be investigated!

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Chapter 8
Drug–Drug Combinations

John Turnidge

Abstract Antimicrobial combination therapy has a long history. The potential for synergy between two antimicrobial agents has been sought using in vitro techniques such as disk approximation, checkerboard titration, in vitro killing experiments with fixed drug concentrations, through the use of in vitro pharmacodynamic models, through animal models, and through retrospective and prospective clinical studies. The most widely examined combinations are those that include aminoglycosides, especially for the treatment of serious *Pseudomonas aeruginosa* infections, and for the management of enterococcal and staphylococcal endocarditis. The in vitro and animal studies of *P. aeruginosa* support the concept that combinations of aminoglycosides with β-lactams improve efficacy and outcomes. Some models suggest that the mechanism of improved efficacy relates less to synergy and more the prevention of the selection of aminoglycoside-resistant mutants. However, human clinical studies to date have failed to demonstrate convincingly that there are better outcomes with combination therapy in terms of efficacy or the prevention of resistance emergence. For endocarditis, there has been strong evidence accumulated for combination therapy enterococcal endocarditis using in vitro and animal models, although there are no randomised clinical data to confirm these. Data supporting the use of combination in staphylococcal therapy has been less robust, and

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the role of combination treatment for this indication is now in question. Ultimately, an in vitro or animal model of the pharmacodynamic interaction of drug classes that can be shown to predict clinical outcomes is still required. Such a model does not currently exist.

Keywords Combination • Synergy • Pseudomonas • Enterococcus • Endocarditis • β-lactam • Aminoglycoside

Introduction

Combinations of antimicrobial agents have a long history in the management of infectious diseases, going back to the days soon after penicillin first became available in the 1940s, when streptomycin was introduced into clinical practice (Selwyn 1983). Even today, combination regimens are commonly used in initial empirical treatment regimens for many infections in order provide sufficient spectrum to treat the possible pathogens pending the results of investigations.

However, what is more important is to develop an understanding of the interaction between two or more antimicrobial agents used for directed therapy against a single pathogen. In this context, combination therapy can have one or more stated aims. These are summarised in Table 8.1. This chapter attempts to examine the evidence that combinations will achieve these aims, including in vitro data, in vitro pharmacodynamic models, animal models and clinical studies. The focus is primarily on antibacterials, although the principles espoused have logical extensions into antivirals and antifungal agents. This chapter does not address certain combinations,

Table 8.1 Rationale for drug combinations

Rationale	Target	Example
"Synergy"	Gram-negative bacteria	β-lactam + aminoglycoside β-lactam + fluoroquinolone Trimethoprim–sulfamethoxazole
Inhibition of degrading bacterial enzymes	Gram-negative bacteria *Staphylococcus* species	β-lactam + β-lactamase inhibitor
Inhibition of degrading human enzymes	Gram-negative and Gram-positive bacteria	Imipenem + cilastatin
Reduced toxicity from reduced dosage	*Cryptococcus neoformans*	Amphotericin B + flucytosine
Broadening of spectrum	Polymicrobial infections	β-lactam + aminoglycoside
Prevention of resistance selection during treatment	*Pseudomonas aeruginosa* *Mycobacterium* species Human immunodeficiency virus	β-lactam + aminoglycoside Combination antituberculous therapy Combination antiretroviral therapy (HAART)
Antibacterial plus antitoxin action	Panton-valentine leukocidin producing *S. aureus*	Cell-wall active agent + clindamycin or linezolid

such as β-lactam: β-lactamase inhibitor combinations, or combinations where the desired interaction is purely pharmacokinetic. The literature on combinations is vast; however, the principles in all cases are similar. The focus of this chapter is to examine in detail two important examples where combination therapy is widely used and recommended: *Pseudomonas aeruginosa* infections and endocarditis.

Combinations for Therapeutic Synergy

Synergy between two antibacterials has been a long-held aspiration in two main clinical settings: serious *Pseudomonas aeruginosa* infections and endocarditis. The use of combinations, especially β-lactams with aminoglycosides is still widely recommended for these two indications.

In Vitro Methods for Synergy Testing

Methods of testing for synergy (also called synergism) in vitro have a long history and have been published in detail by Eliopoulos and Moellering (1996). Qualitative techniques include the use of disk diffusion for both agents, where disks of two agents are placed close to each other on an agar plate seeded with the strain being tested. Synergy manifests itself as a "keyhole" or broadening of the zones of inhibition towards each other. The method suffers from difficulty in ensuring that synergy is or is not present and depends entirely on the antimicrobial contents of each disk, and their proximity to each other.

More often, synergy is determined in the laboratory using the so-called checkerboard technique. This is usually conducted in 96-well microtitre trays. Twofold dilution series of each antimicrobial are made in the horizontal and vertical directions on the plate, resulting in a large array of combinations of antimicrobial concentrations. After inoculation with the test organism and overnight incubation, growth or absence of growth are observed in each well. The pattern of inhibition observed can be used to construct a so-called isobologram, whose pattern can distinguish between synergy, indifference (no interaction) and antagonism between the two agents. While isobologram patterns are qualitative in nature, a more quantitative assessment is often made using the fractional inhibitory concentration (FIC) index first described by Elion et al. (1954). The most commonly accepted quantitative definition of synergy is an FIC index of <0.5 (Eliopoulos and Moellering 1996). Although the interpretation of the FIC index looks straightforward, various definitions have been used over the years, which can lead to wide disparity in detecting synergy or otherwise (Bonapace et al. 2002). Recently, a variation in the FIC index has been developed, named the susceptible breakpoint index (Milne and Gould 2010). This index takes into account both the MICs in checkerboard combinations and the breakpoints for susceptibility of the individual agents. Attempts have been

made to take a more sophisticated mathematical approach to drug interactions in vitro (Greco et al. 1995; Boucher and Tam 2006), but these have yet to gain widespread use.

Traditional in vitro time-kill curves have been adapted to detect synergy between antimicrobial agents (Eliopoulos and Moellering 1996). The standard method involves the use of a fixed combination of the two agents. By convention, synergy is defined in this system as ≥100-fold greater reduction (≥2 $\log_{10}$) in colony counts after 24 h compared to either agent alone. Like the checkerboard technique, antagonism, defined as ≥100-fold greater increase in colony counts after 24 h compared to the more active of the two agents alone, can also be detected in this system.

More sophisticated approaches to time-kill methodology and analysis have been developed (Tam et al. 2004; Lim et al. 2008). Such approaches can quantify the extent of the interaction by integration of the observed killing after 24 h across a wide range of combinations of concentrations. These approaches assume that in vitro killing follows the sigmoid exposure–response curve similar to that observed when single agents are examined over a range of concentrations.

Serious *Pseudomonas aeruginosa* Infections

Much of the work on *P. aeruginosa* has been driven by the specific features of this organism: opportunistic infection in the immune compromised host, relative insensitivity to agents even if they are considered active, and the propensity to select for resistance during treatment.

In Vitro Data

The scientific literature is replete with in vitro studies seeking synergy against *P. aeruginosa* with different drug combinations. Scores of in vitro studies have been tabulated by Eliopoulos and Moellering (1996) and many more have been published since then. Recent studies have focussed appropriately on combinations where the strains being tested might be classified as resistant to one or both agents (Kanellakopoulou et al. 2008; Pankey and Ashcraft 2003; Fujimura et al. 2009; Dundar and Otkun 2010). Typically, the results of these studies show the full range of results from synergy to indifference to antagonism, which will vary from strain to strain for any given combination. With few exceptions, these studies have shown it has not been possible to generalise from the results observed in a single strain to the species as a whole. This would suggest that when synergy is being sought, each strain must be tested individually. However, even when this has been done, it has not been possible to show a consistent correlation with clinical outcome (Chandrasekar et al. 1987; Aaron et al. 2005; Foweraker et al. 2009).

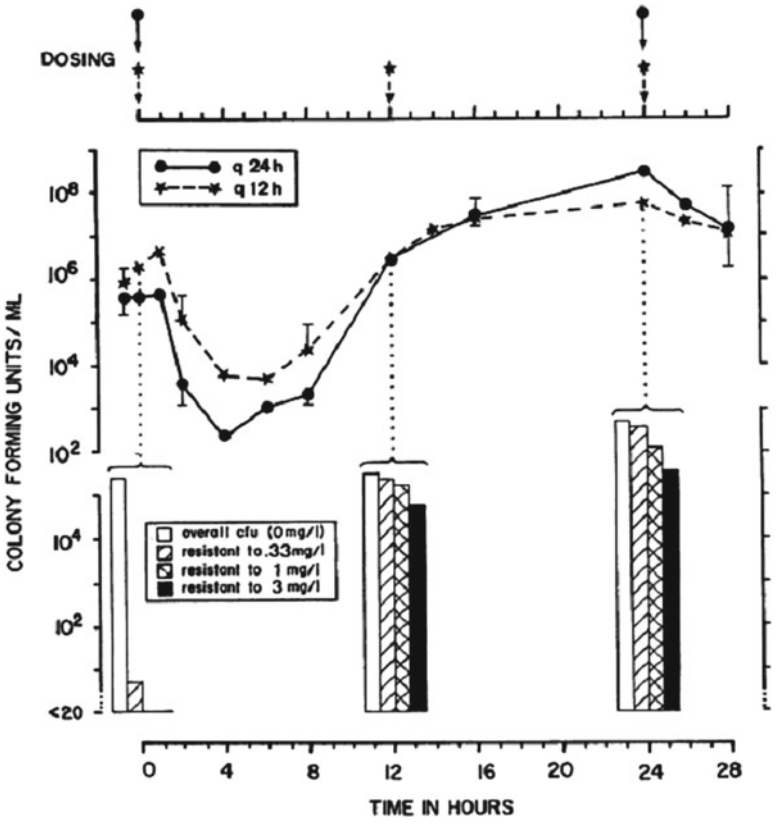

Fig. 8.1 Emergence of resistance to enoxacin in *P. aeruginosa* in an in vitro pharmacodynamic model (from Blaser et al. 1987)

In Vitro Pharmacodynamic Models

In vitro models mimicking the human pharmacokinetics of the recommended doses of test agents have yielded more useful information than conventional in vitro synergy tests. They overcome the problem of fixed drug concentrations and come closer to the situation that might be observed in human plasma with repeated dosing, if not at the site of infection. Their other advantage is they can be extended to periods well beyond the 24 h of a conventional in vitro synergy test. The sentinel in vitro pharmacodynamic model studies were those published in 1987 by Blaser et al. (1987) and Dudley et al. (1987). These investigators were the first to demonstrate, using this type of model, that selection of resistant mutants in *P. aeruginosa* is a common phenomenon (in the absence of host factors) with quinolones [enoxacin (Fig. 8.1) and ciprofloxacin] and aminoglycosides (netilmicin), and that the phenomenon occurs within 24 h of initial dosing.

Aminoglycoside-resistant subpopulations seem to be a natural feature of *P. aeruginosa* (Gerber and Craig 1982). Indeed, resistant subpopulations to important drug classes seem to characterise this species (Hansen et al. 2006; MacGowan et al. 2003; Giwercman and Høiby 1991; Bergen 2008) although their selection during exposure seems to vary considerably, depending particularly on strain and treatment regimen. Presumably selection for resistance during exposure is driven by the extensive suite of intrinsic mechanisms in this species, which can readily confer resistance through upregulation or mutation (Livermore 2002).

As described in Table 8.2, pharmacodynamic models of combination treatment of this species have shown that:

1. Combinations of β-lactams with aminoglycosides prevent the selection of aminoglycoside-resistant mutants (Blaser et al. 1985; McGrath et al. 1993; Zelenitsky et al. 1998) and β-lactam-resistant mutants (Drusano et al. 2012), or both (Zinner et al. 1986).
2. Combinations of β-lactams with fluoroquinolones prevent the emergence of fluoroquinolone-resistant mutants (Lister et al. 2006; Louie et al. 2010), combinations of β-lactams with colistin prevent the emergence of fluoroquinolone-resistant mutants (Gunderson et al. 2003; Bergen et al. 2011).
3. The dosing regimen of aminoglycosides has varying effects on the risk of resistance selection (Blaser et al. 1987; Zelenitsky et al. 1998).
4. The combination of a carbapenem with an aminoglycoside or fluoroquinolone can be effective in killing and preventing resistance emergence even for strains that are not susceptible to one or both agents (Lister et al. 2006); similar observations have been made with colistin and a carbapenem for a colistin-resistant strain (Bergen et al. 2011).
5. Killing can occur even when the combination of a β-lactam and aminoglycoside are below the MICs throughout (den Hollander et al. 1997).
6. Aztreonam can enhance the efficacy of cefepime but not ceftazidime (Lister et al. 1998).
7. With the combination of tobramycin and ceftazidime, the parameter that best predicts efficacy is percentage time above a parameter described by the in vitro inhibitory concentrations of both agents (den Hollander et al. 1998).
8. There is conflicting evidence about the superiority or inferiority of staggering the administration of β-lactams when combined with either aminoglycosides or fluoroquinolones (Guggenbichler et al. 1988; Dudley et al. 1991; Barclay et al. 1995; Zelenitsky et al. 2004).

Although these studies have been very helpful in approaching an understanding of combination treatment, there are a number of limitations in many of them. All in vitro models necessarily ignore the effects of host factors on antimicrobial efficacy. Sometimes, studies mimicked the agent's kinetics without taking into account the effect of protein binding, i.e. they have modelled total plasma concentrations, rather than free concentrations. Finally, many studies only examine bacterial counts over the first 24 h of exposure. Two of the referenced studies used the hollow fibre model, which makes following the pharmacodynamics over long periods easier (Drusano et al. 2012; Louie et al. 2010). These studies clearly showed the benefits of prolonged drug exposure and sampling over periods commensurate with those used in clinical practice.

Table 8.2 In vitro pharmacodynamics studies of antimicrobial combinations against *Pseudomonas aeruginosa*

Reference	Combination(s) examined	Primary finding(s)	Secondary finding
Blaser et al. (1985)	Netilmicin–ceftazidime	• Regrowth over 24 h with netilmicin whether given once-daily or in three divided doses 8-hourly • Ceftazidime given by continuous infusion prevented regrowth	• More rapid killing with netilmicin compared to ceftazidime
Zinner et al. (1986)	Amikacin–azlocillin	• Combination prevented regrowth seen with either agent used alone	
Guggenbichler et al. (1988)	Gentamicin–ticarcillin	• Staggered administration of the two agents resulted in greater bacterial killing compared to simultaneous administration	
Dudley et al. (1991)	Ciprofloxacin–azlocillin	• Simultaneous administration produced greater killing than when drug dosing of the two agents was staggered (either agent first) • Prevention of resistance to either agent when used in combination	
McGrath et al. (1993)	Amikacin–imipenem	• Regrowth over 24 h with amikacin whether given once-daily or in two divided doses 12-hourly • Imipenem given 8- to 12-hourly prevented regrowth	• Resistant subpopulations to imipenem are present normally present, but unlike with amikacin are not amplified following imipenem exposure
Barclay et al. (1995)	Gentamicin–ceftazidime	• Simultaneous administration results in greater bacterial killing than when dosing is staggered	
den Hollander et al. (1997)	Tobramycin–ceftazidime	• Combinations that included once-daily tobramycin result in greater killing	• Killing can be achieved even when both agents are below the MIC throughout
Zelenitsky et al. (1998)	Tobramycin–ceftazidime Tobramycin–ciprofloxacin Tobramycin–imipenem	• More rapid killing with combinations compared to tobramycin 8- or 24-hourly • Combinations prevented regrowth with the tobramycin 24-hourly regimen	• Regrowth not observed with tobramycin 8-hourly

(continued)

Table 8.2 (continued)

Reference	Combination(s) examined	Primary finding(s)	Secondary finding
den Hollander et al. (1998)	Tobramycin–ceftazidime	• "Synergistic" action even when concentrations of both agents fall below MICs • Best predictor of efficacy is the calculated parameter "T > FICi"	• The FICi calculated from checkerboard or Etest synergy studies
Lister et al. (1998)	Cefepime–aztreonam Ceftazidime–aztreonam	• Enhancement of cefepime efficacy by aztreonam, especially for chromosomal cephalosporinase de-repressed strains	• No enhancement of ceftazidime efficacy by aztreonam
Gunderson et al. (2003)	Colistin–ceftazidime Colistin–ciprofloxacin	• Enhancement of colistin efficacy by continuous infusion ceftazidime • No enhancement of colistin activity by ciprofloxacin	
Zelenitsky et al. (2004)	Ciprofloxacin–ceftazidime Tobramycin–ceftazidime	• Better killing at 48 h when agents given simultaneously or ceftazidime is given first	
Lister et al. (2006)	Levofloxacin–imipenem	• Imipenem prevented emergence of levofloxacin resistance even with imipenem non-susceptible strains	
Louie et al. (2010)	Levofloxacin–meropenem	• Monotherapy selects for resistance over 14 days of exposure except at supra-maximum dosing regimens • Combination treatment with standard dosage regimens prevents the emergence of resistance to either agent	• Findings hold true even for a MexAB pump-over-expressing mutant
Bergen et al. (2011)	Colistin–doripenem	• Colistin-resistant subpopulations substantially reduced by combination treatment • Combinations for most dosing regimens had greater efficacy than colistin alone, depending on strain tested	
Drusano et al. (2012)	Tobramycin–cefepime	• Tobramycin effective suppressed the selection of cefepime-resistant mutants during prolonged exposure	

Animal Model Data

Studies of combination treatments of *P. aeruginosa* infection in animal models have been conducted since the early 1980s. Although there has been considerable optimism that animal models would hold to key to demonstrating true synergy in vivo (Fantin and Carbon 1992), the findings of the early studies are at best indicative because they precede our full understanding of antimicrobial pharmacodynamics and its associated targets (in the late 1980s).

The goals of animal models are several, but principally animal model studies attempt to validate the observations of static concentrations in vitro or in vitro pharmacodynamic models. Some animal model studies have attempted to compare conventional interaction assays, such as the checkerboard assay or time-kill curves, with combination treatment outcomes in animal models, using endpoints such as change in bacterial loads at the site of infection, and/or mortality. Interpretation of many of these studies though is difficult for a range of reasons related to study design, including some or all of the following:

1. Outcome evaluation quite soon after antimicrobial exposure (hours to 1–2 days); this is not long enough to know whether there are realisable benefits over longer courses of treatment used in humans.
2. Antimicrobial exposures different from those that would be observed in humans.
3. Induced neutropenia to allow a focus on antibacterial effects by eliminating the main host defence; models that include this in their design will inform treatment in neutropenic patients, but their applicability in the non-neutropenic setting is unclear.
4. Failure to account for differences in protein binding between the species of animal being used and humans.
5. Failure to consider the emergence of less susceptible and resistant subpopulations during treatment; this is a particular feature of this species.

Despite these potential limitations, animal models have been informative in a number of ways. Probably, the most important initial studies were those published in a series of landmark papers in 1982 by Gerber, Craig, and co-workers (Gerber et al. 1982, 1983; Gerber and Craig 1982). Employing the now widely used neutropenic mouse thigh model, these investigators demonstrated for the first time that selection of aminoglycoside (gentamicin)-resistant variants occurs in vivo and that this can be largely prevented by combining with a β-lactam (ticarcillin) (see Fig. 8.2). Unfortunately, these studies received little attention initially and many animal model studies have subsequently been performed without recognising these critical features of *P. aeruginosa* animal models (Table 8.3).

A summary of the most reproducible findings from the studies (listed in Table 8.3) that followed the landmark papers is provided below. The usual endpoints that led to these conclusions were significant reduction in bacterial load and/or mortality.

1. Combinations that are generally better than either of the agents administered singly.

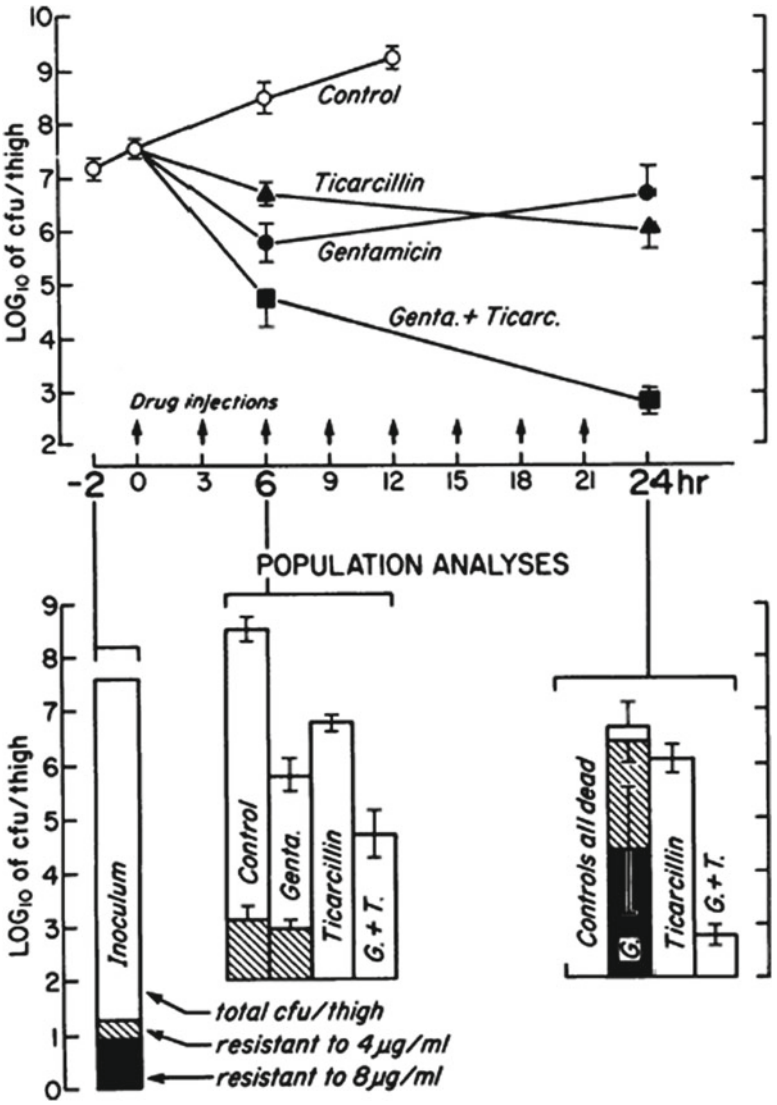

Fig. 8.2 Selection of resistance during treatment and suppression by combination therapy (from Gerber et al. 1982)

(a) β-Lactams with aminoglycosides (Peterson et al. 1984; Rusnak et al. 1984; Bayer et al. 1985a; Mordenti et al. 1985; Johnson 1985; Chin et al. 1986; Chadwick et al. 1986; Moody et al. 1987; Gordin et al. 1987; Gerber et al. 1989; Ulrich et al. 1989; Pefanis et al. 1993; Mimoz et al. 1999; Robaux et al. 2001; Placensia et al. 2007; Chan et al. 2006; Maiques et al. 2007; Yuan et al. 2010); although there are exceptions (Ulrich et al. 1989;

Table 8.3 In vivo pharmacodynamics studies of antimicrobial combinations against *Pseudomonas aeruginosa*

Reference	Animal model	Combination(s) examined	Primary finding(s)	Duration of treatment
Chusid et al. (1983)	Normal and neutropenic guinea pig septicaemia	Tobramycin–ticarcillin	• No evidence of synergy; combination reduced bacterial load to the same extent as single agents	40 h
Peterson et al. (1984)	Rabbit tissue chamber	Amikacin–azlocillin Amikacin–ceftizoxime Amikacin–cefoxitin	• Single agent treatment did not eliminate any of six test organisms • Azlocillin–amikacin eliminate 5/6 organisms • Ceftizoxime–amikacin eliminated 3/6 organisms • Combinations of β-lactams had not effect	96 h
Zuravleff et al. (1984)	Neutropenic mouse septicaemia	Tobramycin–ticarcillin–rifampin	• Rifampin added to the ticarcillin–tobramycin combination significantly enhanced survival	120 h
Rusnak et al. (1984)	Neutropenic guinea pig pneumonia	Tobramycin–ticarcillin Tobramycin–ceftazidime Tobramycin–azlocillin Netilmicin–ceftazidime	• Combinations gave greater reduction in bacterial load compared to agents used alone • Combinations gave only a small improvement in mortality	36 h
Bayer et al. (1985a)	Rabbit endocarditis	Amikacin–ceftazidime	• Greater activity of the combination in terms of mortality and reduction of the bacterial burden • Resistant strains very common after 14 days of amikacin monotherapy but uncommon with ceftazidime monotherapy • Combinations reduced emergence of amikacin resistance	
Mordenti et al. (1985)	Neutropenic rat peritonitis	Amikacin–ticarcillin	• Amikacin plus ticarcillin administered by continuous infusion gave best results in terms of bacterial killing and mortality at 72 h, compared to any combination of intermittent infusion of both agents • Mortality was highest when drugs were used alone	96 h
Bayer et al. (1985b)	Rabbit endocarditis	Amikacin–ceftazidime	• Greater activity of combination compared to single agents at 7 but not 14 days of therapy	7–14 days

(continued)

Table 8.3 (continued)

Reference	Animal model	Combination(s) examined	Primary finding(s)	Duration of treatment
Johnson (1985)	Neutropenic rat bacteraemia	Amikacin–imipenem Amikacin–moxalactam Amikacin–piperacillin Amikacin–ticarcillin Amikacin–ceftazidime Ceftazidime–piperacillin	• Amikacin combined with either imipenem or ceftazidime gave the best results in terms of 96-h bacteraemia clearance and mortality, followed by amikacin plus ticarcillin and piperacillin • Single agent therapy had low efficacy and high mortality, apart from imipenem	~72 h
Chin et al. (1986)	Neutropenic mouse peritonitis	Ciprofloxacin–azlocillin	• Reduced mortality with combination treatment at day 7 (4 days post-treatment) compared to single agent treatment	72 h
Kemmerich et al. (1986)	Guinea pig pneumonia	Ciprofloxacin–azlocillin Ciprofloxacin–ceftazidime Ciprofloxacin–tobramycin	• Reduction in bacterial load in the lung not enhanced by combinations compared to ciprofloxacin alone	Single doses
Péchere et al. (1986)	Mouse peritonitis	Amikacin–ceftriaxone Amikacin–pefloxacin Pefloxacin–ceftriaxone	• Ready selection of resistant mutants to all three agents when used alone • Combinations reduced but did not always eliminate the resistant mutant selection	Single doses
Johnson and Thompson (1986)	Neutropenic rat bacteraemia	Amikacin–azlocillin Amikacin–ticarcillin	• Combination of amikacin with azlocillin, but not ticarcillin, prevented the selection of resistant mutants	62 h
Chadwick et al. (1986)	Neutropenic rat bacteraemia	Amikacin–imipenem	• Mortality much reduced with combination compared to either agent alone	48 h
Moody et al. (1987)	Rabbit tissue chamber	Amikacin–ceftazidime	• Combination was successful in clearing organisms in 5 of 6 strains, compared to none treated with ceftazidime alone	96 h
Johnson et al. (1987)	Rat septicaemia	Ciprofloxacin–tobramycin Ciprofloxacin–azlocillin Tobramycin–azlocillin	• Compared to each of the agents used alone, mortality was reduced with the ciprofloxacin combinations, but not with the tobramycin–azlocillin combination	60 h
Gordin et al. (1987)	Guinea pig pneumonia	Tobramycin–ceftazidime	• Greater clearance of bacteria from the lungs with the combination compared to either agent alone	36 h

Thauvin et al. (1989)	Rat endocarditis	Ciprofloxacin–azlocillin Tobramycin–azlocillin	• Azlocillin combinations were not superior to single agent treatment in terms of mortality or reduction in the bacterial burden • Ciprofloxacin was superior to tobramycin	6 days
Gerber et al. (1989)	Neutropenic mouse thigh	Netilmicin–azlocillin	• Combination had greater killing than azlocillin alone • Once-daily had greater killing than thrice-daily netilmicin in two of three strains tested	32 h
Ulrich et al. (1989)	Neutropenic mouse septicaemia	Ciprofloxacin–azlocillin Tobramycin–imipenem	• Combinations gave greater survival than single agent treatment for only one of the two strains tested • Azlocillin did not protect against the selection of decreased susceptibility to ciprofloxacin	96 h
Froidefond et al. (1992)	Mouse peritonitis	Ciprofloxacin–imipenem Ciprofloxacin–ceftazidime Amikacin–imipenem Amikacin–ceftazidime	• Major reduction in resistance selection when β-lactam combined with ciprofloxacin or amikacin	8 h
Gudmundsson et al. (1993)	Neutropenic mouse thigh	Tobramycin–imipenem Tobramycin–ceftazidime Imipenem–rifampin Tobramycin–rifampin Imipenem–tobramycin–rifampin Ceftazidime–tobramycin–rifampin	• Overall, combinations tended to significant prolong the post-antibiotic effect compared to agents used alone	Single doses
Pefanis et al. (1993)	Rabbit endocarditis	Amikacin–ceftazidime Amikacin–aztreonam	• For a relatively susceptible strain, combinations were not superior in terms of survival compared to single agents • For a strain resistant to aztreonam and amikacin, both combinations increased survival	10 days
Bugnon et al. (1997)	Rabbit endocarditis	Pefloxacin–fosfomycin	• Fosfomycin more effective in reducing bacterial load than pefloxacin • Pefloxacin antagonised the fosfomycin effect	24 h

(continued)

Table 8.3 (continued)

Reference	Animal model	Combination(s) examined	Primary finding(s)	Duration of treatment
Mouton et al. (1999)	Neutropenic mouse thigh	Tobramycin–ticarcillin Netilmicin–ceftazidime Ciprofloxacin–ceftazidime Ciprofloxacin–netilmicin	• Bacterial killing best predicted by the sums of the responses of the single-agent regimens as functions of their respective pharmacodynamic indices • Analysis suggested that combinations were "synergistic". However this conclusion was based on using linear regression rather than modelling the Hill function for response	24 h
Mimoz et al. (1999)	Rat pneumonia	Amikacin–imipenem Amikacin–piperacillin–tazobactam Amikacin–cefepime	• All combination treatments resulted in greater reduction in bacterial burden in the lung than any of the agents alone • Piperacillin–tazobactam plus amikacin resulted in greater bacterial killing than amikacin alone, even though the strain appeared to be resistant to piperacillin–tazobactam	60 h
Robaux et al. (2001)	Rabbit endocarditis	Amikacin–ceftazidime	• Reduction in bacterial counts was generally better with continuous rather than intermittent dosing of ceftazidime • Combination with amikacin resulted in additional killing for only two of four strains, and only for one of the two ceftazidime dosing regimens applied to those strains	24 h
Navas et al. (2004)	Rabbit endocarditis	Tobramycin–imipenem Tobramycin–cefepime	• Tobramycin dosing mimicking that of 3 mg/kg daily in humans did not enhance the killing of bacteria in vegetations	24 h
Maciá et al. (2006)	Mouse chronic lung infection	Ciprofloxacin–tobramycin	• Superior efficacy (lower mortality) with combination compared either agent alone • No effect of tobramycin on mortality compared to placebo • Combination prevent the emergence of resistance for the standard strain and its hypermutable variant	72 h
Placensia et al. (2007)	Mouse chronic lung infection	Tobramycin–ceftazidime Ciprofloxacin–ceftazidime Ciprofloxacin–tobramycin	• Resistance selection with all single agents alone with a hypermutable derivative but not parent strain (CIP>CTZ>TOB) • All combinations greatly reduced the bacterial load compared to single agents and prevented resistance emergence for parent	24 h

Chan et al. (2006)	Neutropenic rat sepsis	Amikacin–piperacillin	• Combination gave greater bacterial killing at 5 h than either agent alone • Described differences in killing depending on the dosing ratio for the two agents	Single doses
Maiques et al. (2007)	Guinea pig meningitis	Tobramycin–ceftazidime	• Bacterial killing equivalent at 8 h for ceftazidime compared to combination with tobramycin • Combination superior in terms of negative culture rates at 48 h	Single dose and 48 h
Cirioni et al. (2007)	Neutropenic rat peritonitis/sepsis	Colistin–rifampin	• Despite minimal effects of rifampin alone, the combination enhanced the effect of colistin in terms of mortality and bacterial load reduction	Single doses
Croisier et al. (2008)	Rabbit pneumonia	Tobramycin–ceftazidime	• Modest reduction of bacterial load in lungs for all single regimens and combination regimens • Significant reduction of bacterial burden in spleens with continuous infusion ceftazidime either alone or in combination with tobramycin, when compared to other treatments but not between themselves	48 h
Aoki et al. (2009)	Mouse pneumonia	Colistin–rifampin Colistin–imipenem	• In terms of survival, combinations were no better than single agents, unless the colistin was administered intranasally rather than subcutaneously	48 h
Yuan et al. (2010)	Neutropenic mouse pneumonia	Amikacin–cefepime Levofloxacin–cefepime Amikacin–levofloxacin	• Greatest reduction in burden at 24 h with amikacin–cefepime, followed by levofloxacin–cefepime • Amikacin–levofloxacin burden not different from untreated controls • Similar comparison for 96 h mortality	Single doses

Pefanis et al. 1993; Robaux et al. 2001; Johnson et al. 1987; Chusid et al. 1983; Kemmerich et al. 1986; Thauvin et al. 1989; Johnson and Thompson 1986; Navas et al. 2004), many of which were strain dependent and/or were seen in endocarditis models.

(b) β-Lactams with fluoroquinolones (Ulrich et al. 1989; Johnson et al. 1987; Placensia et al. 2007), with some exceptions (Ulrich et al. 1989; Thauvin et al. 1989).

2. Combinations of fluoroquinolones with aminoglycosides gave variable outcomes from no better than at least one of the agents administered singly (Johnson et al. 1987; Yuan et al. 2010), or better than either of the single agents (Placensia et al. 2007; Maciá et al. 2006).
3. Selection of resistance to aminoglycosides can be reduced or eliminated by concomitant agents including:

   (a) β-Lactams (Bayer et al. 1985a; Placensia et al. 2007; Péchere et al. 1986; Johnson and Thompson 1986; Froidefond et al. 1992) with some exceptions (Johnson and Thompson 1986).
   (b) Fluoroquinolones (Placensia et al. 2007; Péchere et al. 1986).

4. Selection of resistance to fluoroquinolones can be reduced or eliminated by concomitant agents including:

   (a) β-Lactams (Placensia et al. 2007; Froidefond et al. 1992).

5. Continuous infusion of β-lactams combined with other classes gives better results than intermittent dosing (Mordenti et al. 1985; Robaux et al. 2001; Croisier et al. 2008).
6. Once-daily administration of aminoglycosides is usually more effective than thrice-daily (Gerber et al. 1989).
7. Combinations of aminoglycosides and β-lactams prolong the in vivo post-antibiotic effect compared to the agents alone (Gudmundsson et al. 1993).
8. Colistin combinations give variable effects from improved survival (Cirioni et al. 2007) to no benefit when the colistin is administered systemically (Aoki et al. 2009).
9. Rifampin in high doses can enhance the effect of other active agents alone or in combination (Gudmundsson et al. 1993; Cirioni et al. 2007; Zuravleff et al. 1984).
10. Occasionally, true antagonism can be demonstrated (Bugnon et al. 1997).

Duration of treatment is an important consideration, but only two studies went beyond 7 days to test efficacy. The first of these, a rabbit endocarditis study showed a benefit in reducing bacterial load at 7 days that disappeared when treatment was continued for 14 days (Bayer et al. 1985a). The second study, using the same model, showed a benefit of combination therapy after 10 days of treatment, but only for a strain relatively resistant to both test agents (amikacin and aztreonam) (Pefanis et al. 1993).

There has been only one serious attempt to define the PK/PD parameters in animal models that define efficacy (Mouton et al. 1999). In a detailed analysis of single and combination agent data from the neutropenic mouse thigh model, Mouton and colleagues proposed that the best predictor of bacterial killing in vivo was the sum of responses of the single-agent regimens as a function of their respective pharmacodynamic indices, which were $T>(0.25\times)$MIC for ticarcillin and ceftazidime, and AUC/MIC ratio tobramycin, netilmicin and ciprofloxacin. For instance, if a ticarcillin treatment regimen resulted in a percentage time above MIC that produced a 1 $\log_{10}$ kill after 24 h, and a tobramycin treatment regimen resulted in an AUC/MIC ratio that also produced a 1 $\log_{10}$ kill after 24 h, the expected kill when the regimens were combined would be 2 $\log_{10}$. Because this combined effect is greater than that observed by either agent administered alone, this could be interpreted as describing "synergy" in vivo.

Clinical Studies

Efficacy of Combinations

Despite the wealth of data generated by in vitro and animal model studies, uncertainties still exist about their clinical relevance and therefore the utility of antibacterial combinations for treating *P. aeruginosa* infections, particularly the more serious ones (van Delden 2007). Clinical practice recommendations such as those made by the Sanford guide (Gilbert et al. 2011) are currently in favour of combinations for more serious *P. aeruginosa* infections, with a strong emphasis on the combination of a β-lactam and either an aminoglycoside and/or a fluoroquinolone with antipseudomonal activity.

Evidence from clinical studies to supporting the concept of combination therapy producing better clinical outcomes is unfortunately scant. In a recent Cochrane meta-analysis, Paul et al. showed no better outcomes (clinical success) with combination aminoglycoside therapy for *P. aeruginosa* sepsis over treatment with a β-lactam alone (Paul et al. 2009). The analysis included cases from 6 studies where the same β-lactam was used in each arm, and 12 studies where the β-lactam was different in each arm. In summary, the risk ratio for clinical failure with combination therapy was 1.02 (CI = 0.68, 1.51) for studies using the same β-lactam, and 1.24 (CI = 0.77, 1.98) for studies using a different β-lactam. Total numbers in either arm of the 18 studies were not large (222 monotherapy, 204 combination therapy) decreasing the sensitivity of detecting a difference. Another factor reducing sensitivity is the inclusion of patients with likely good outcomes due to source control, as pointed out by van Delden (2007).

The Cochrane meta-analysis is countered to some extent by another meta-analysis that examined the studies using combinations more generally for Gram-negative bacteraemia (Safdar et al. 2004). In a sub-analysis of five studies which compared outcomes of single agent and combination agent therapy specifically for

P. aeruginosa bacteraemia (Tapper and Armstrong 1974; Hilf et al. 1989; Mendelson et al. 1994; Siegman-Igra et al. 1998; Kuikka and Valtonen 1998), the authors concluded that combination therapy was significantly better than monotherapy with an odds ratio of 0.50 (CI 0.32–0.79) for mortality. These findings are reinforced by those of Chatzinikolaou et al. (2000) who, while they did not report mortality figures, showed a clinical cure rate of pseudomonal bacteraemia in cancer patients of 50.5 % in patients receiving monotherapy and 68.4 % in patients receiving combination therapy in the initial regimen ($p=0.0001$). Furthermore, Chamot et al. were able to show a difference in 30-day mortality in patients who received adequate monotherapy compared to those who received adequate combination therapy as initial empirical treatment (hazard ratio of 3.7; CI = 1.0, 14.4; $p=0.05$) (Chamot et al. 2003). For their analysis, the investigators defined "adequate monotherapy" as single drug therapy with a β-lactam or a fluoroquinolone (ciprofloxacin), but not an aminoglycoside (gentamicin), and "adequate combination therapy" was defined as two-drug therapy with any combination of a β-lactam, an aminoglycoside or a fluoroquinolone to which the infecting strains was susceptible. The other relevant finding from this study was that there was no significant difference between monotherapy and combination therapy when used as definitive treatment, that is, when culture and susceptibility test results became available.

One important difference between the two meta-analyses was that the first involved infections with any kind of "serious" *P. aeruginosa* infection, not necessarily bacteraemic ones, while the latter exclusively focussed on bacteraemic infection. Another difference with the second meta-analysis was that monotherapy involved a non-β-lactam in the majority of cases, mainly an aminoglycoside. Indirectly, this difference suggests that aminoglycosides, while capable of more rapid bactericidal activity initially, are inferior to β-lactams as monotherapy. However, there are a number of caveats with the five studies included in this meta-analysis (1) drug doses and regimens were not documented in any of the studies and (2) three studies involved or included cases in the 1970s, before we had a better understanding of aminoglycoside pharmacodynamics and were applying it in clinical practice.

There has been a recent attempt to summarise the available comparative efficacy data from reviews and other studies for pneumonia, mainly hospital associated and ventilator associated, caused by *P. aeruginosa* (Sun et al. 2011). Even for this specific condition, the commonest conclusion was that combination therapy did not produce superior efficacy. Only one cited study focussed specifically on *P. aeruginosa* as a cause of pneumonia (Garnacho-Montero et al. 2007). This multicentre study, examining 183 episodes of pneumonia, showed that there was a benefit of initial combination therapy, but only in terms of ensuring the administration of one active agent. If the isolate was susceptible to at least one agent, then combination therapy was not better than monotherapy in effecting cure.

Of interest, one of the studies included in the second meta-analysis (Hilf et al. 1989) attempted to correlate the results of both checkerboard and time-kill in vitro studies with outcome (mortality). The demonstration of synergy by either in vitro method did not correlate with lower mortality. Thus, we are left with the conclusion that despite intensive efforts to demonstrate the therapeutic benefits of antimicrobial

combinations for the treatment of serious *P. aeruginosa* infections using in vitro and animal models, clinical correlates are not as strong as might be hoped, with the possible exception of *P. aeruginosa* bacteraemia. It may well be that the benefit of combination therapy is for initial empirical therapy where there is a reasonable risk of the infection being caused by *P. aeruginosa*.

However, it would be inappropriate to dismiss the role of definitive combination therapy in producing better clinical outcomes for at least some serious *P. aeruginosa* infections based on currently published clinical studies. This evidence base is less than optimum for a number of reasons. Firstly, most of the clinical data have been generated from retrospective analyses. Secondly, the dosing regimens used may not have reached pharmacodynamic targets because many standard antipseudomonal regimens have been developed prior to the application of pharmacodynamic principles, and also because many critically ill patients have augmented renal clearance and higher than expected volumes of distribution (Varghese et al. 2011). Thirdly, pooling of agents into monotherapy and combination therapy for the purposes of analysis in these studies may mask the pharmacodynamic benefits of specific combinations. Fourthly, only one study has addressed the more complex effects on outcome of empirical versus definitive therapy (Chamot et al. 2003), which clearly impacts on outcome. Finally, different outcomes are expected for different types of serious *P. aeruginosa* infection (van Delden 2007). Mortality will clearly be highest when septic shock is present (Chamot et al. 2003; Vidal et al. 1996; Kang et al. 2003) while bacteraemia secondary to surgery and pneumonia have higher mortality (Chatzinikolaou et al. 2000; Chamot et al. 2003; Vidal et al. 1996) than bacteraemia when it has taken origin from the urinary tract or intravascular lines (Chamot et al. 2003).

Cystic fibrosis is a special example of *P. aeruginosa* infection with persistent and chronic airway infection punctuated by acute exacerbations and is associated with mucoid (alginate-producing) strains. Antipseudomonal therapy for acute exacerbations is standard for this condition. Many studies comparing single versus combination antipseudomonal therapy have been conducted and subjected to repeated meta-analysis as part of the Cochrane Database program. Outcome measures for this infection are different from those of other *P. aeruginosa* infection. The most recent meta-analysis by Elphick and Tan concluded that there was insufficient evidence to support the concept that combination therapy was superior to monotherapy in terms of lung function; symptom scores; adverse effects and bacteriological outcome measures. However, they noted significant heterogeneity between studies, and that many of the studies of sufficient quality to include in the meta-analysis were conducted more than 20 years ago. Ultimately, they pointed to the pressing need for a blinded prospective study to address the problem.

Prevention of Resistance Emergence During Treatment

The other major potential benefit of combination therapy is the control of resistance emergence during treatment. Here, the evidence is more limited. Early evidence

from lower respiratory infections in patients without neutropenia or cystic fibrosis suggested no benefit from combination therapy in preventing the selection for resistance to β-lactams during treatment (Nichols and Maki 1985). However, this referred to studies focussing on resistance to less potent antipseudomonal β-lactams such as cefsulodin, ticarcillin and carbenicillin. Nevertheless, there were hints from a number of studies, which included small numbers of *P. aeruginosa* that there was a potential benefit (Klastersky et al. 1973; Michalsen and Bergan 1981; Gribble et al. 1983; McLaughlin et al. 1983; Kosmidis and Koatzanis 1986).

Carmeli et al. (1999) monitored for the emergence of resistance in 271 patients infected with *P. aeruginosa* and treated with a variety of antipseudomonal agents. The only significant link between treatment and resistance emergence that they could find was to imipenem, and they were not able to show a protective effect of combination treatment (stated to be combination with aminoglycoside), although combinations were only used in 28 % of patients. Unfortunately, these investigators did not examine for resistance emergence to aminoglycosides, which is suggested by the animal models to be the greater problem, presumably because few or no patients were treated with aminoglycosides alone.

Indirect evidence the combinations of antipseudomonal agents might protect against the emergence of resistance during treatment has been shown in another study that examined the impact of prior antimicrobial therapy on the presence of resistance in a subsequent blood culture isolate of *P. aeruginosa* (Boffi El Amari et al. 2001). Prior combination therapy with antipseudomonal activity, defined as use of two agents from two different classes of any of the β-lactam, aminoglycoside or fluoroquinolone class, was not associated with resistance to either of the agents on multivariate analysis. In contrast, prior monotherapy was associated with resistance, particularly for ceftazidime and imipenem, although notably not for ciprofloxacin or aminoglycosides. However, when prior monotherapy was compared directly to prior combination therapy, no significant difference in rates of resistance to the agents used was detected; this can be attributed to the small number of patients who had received prior combination antipseudomonal agents (3.7 % of the patients studied).

More recently, Bliziotis et al. (2005) conducted a meta-analysis attempting to answer the question of resistance selection during treatment with β-lactam monotherapy versus β-lactam-aminoglycoside combination therapy. Amongst eight eligible studies included in the analysis, only a small number of *P. aeruginosa* isolated were included overall ($n=87$) and rates of resistance emergence with monotherapy (20.5 %) were no different from combination therapy (20.8 %).

Thus, the question of reducing or eliminating the emergence of resistance during treatment using combination agents remains unanswered. Although the theoretical, in vitro and animal model data are suggestive of benefit (Mouton 1999), the true value in clinical practice remains uncertain (DeRyke et al. 2006). Large prospective studies, ideally double-blind and randomised, are required to address the two major questions (1) is combination therapy superior to monotherapy for serious *P. aeruginosa* infections in terms of efficacy and/or the prevention of resistance emergence? and (2) importantly for β-lactam–aminoglycoside combinations, do the risks of toxicity outweigh the benefits?

Endocarditis

Combination therapy for endocarditis has a very long history, beginning in the 1940s (Hunter 1946, 1947; Robbins and Tompsett 1951; Cates et al. 1951). The rationale for the original combination of penicillin and streptomycin in these early cases was "resistance" to penicillin in the infecting pathogen, combined with some in vitro tests that suggested synergy with the combination. This concept is still extant.

The use of combination therapy has been the standard approach to Gram-positive endocarditis for many years, especially for enterococcal, viridans streptococcal and staphylococcal endocarditis. It is widely accepted that treatment of enterococcal endocarditis responds poorly or not at all to single agents in the β-lactam or glycopeptide class, and that where possible an aminoglycoside must be used in combination to ensure a high likelihood of success. For viridans streptococcal endocarditis, combinations are considered standard only when the penicillin MIC is elevated to levels similar to those seen with wild-type *Enterococcus* species. For *Staphylococcus aureus* endocarditis, the recommendation of combination therapy became standard in the 1970s but has recently been brought into question.

By far, the greatest interest in terms of potential synergy in the management of endocarditis has been in combinations of penicillins and aminoglycosides. Comparatively little data are available on other combinations until the advent of methicillin- and multi-resistant strains of *Staphylococcus aureus*, and then the emergence of vancomycin-resistant *Enterococcus* species.

In Vitro Data

The standard laboratory techniques, namely checkerboard and time-kills methods, have been applied to a greater or lesser extent to endocarditis pathogens.

Viridans Streptococci

Interaction between penicillin and aminoglycosides using in vitro methods yields various results that vary from species to species and strain to strain (Jawetz and Gunnison 1950; Duperval et al. 1975; Shanson et al. 1986; Potgieter et al. 1992; Vigliarolo et al. 2007).There are conflicting data about whether the mechanism of synergy in viridans streptococci is related to enhanced uptake of aminoglycoside (Yee et al. 1986; Miller et al. 1986). As is frequently observed with enterococci, high-level "resistance" to aminoglycosides appears to abolish synergy (Farber et al. 1983; Farber and Yee 1987). High-level "resistance" to aminoglycosides is being found in viridans streptococci with increasing frequency (Stevens et al. 1992), although whether the same values used to define high-level resistance to aminoglycosides in enterococci also apply to the viridans streptococci is less clear. Synergy

in vitro has also been demonstrated in vivo for ampicillin (Watanakunakorn and Glotzbecker 1979), amoxicillin (Basker and Sutherland 1977) and glycopeptides combined with aminoglycosides (Shanson and Tadayon 1986).

Enterococci

Compared to most other species, time-kill studies show that enterococci are killed rather slowly in vitro (Glew and Moellering 1979; Ryan et al. 1981; Chen and Williams 1983), and as a result MIBCs are usually much higher than MICs in conventional overnight assays (Chen and Williams 1983). For this reason, and the fact that early experience with penicillin alone, albeit in modest doses by today's standards, was ineffective in treating endocarditis (Hunter 1946, 1947; Robbins and Tompsett 1951; Cates et al. 1951), most in vitro synergy work has focussed on *Enterococcus* species. Synergy in enterococci was demonstrated in vitro between penicillin and streptomycin soon after the reports of successful treatment of enterococcal endocarditis (Jawetz et al. 1950). The mechanism of synergy of this combination on enterococci was later demonstrated by Moellering and Weinberg to be the result of enhanced streptomycin uptake in the presence of penicillin (Moellering and Weinberg 1971). In the absence of acquired resistance mechanism, synergy seems to be a feature of all strains of enterococci (Moellering et al. 1971), although some have suggested that it is not universal (Jawetz and Sonne 1965). This is despite the fact that aminoglycosides usually have poor activity against enterococci, inhibiting them at levels above what would be expected to the effective clinically. Synergy is also seen with other aminoglycosides (Eliopoulos and Moellering 1996), as is synergy between aminoglycosides and other cell-wall active agents such as glycopeptides (Eliopoulos and Moellering 1996). Thus, the increased uptake hypothesis, resulting in sufficient intracellular concentration of aminoglycosides, appears to hold true for the combinations of cell-wall active agents and aminoglycosides generally.

The synergistic action is eliminated when enterococci have acquired aminoglycoside resistance mechanisms and become so-called high-level resistant (Moellering et al. 1970; Standiford et al. 1970), mainly through the acquisition of aminoglycoside-modifying enzymes (Eliopoulos and Moellering 1996). The individual chemical features of each aminoglycoside, and the differing properties of each aminoglycoside-modifying enzyme, result in three groups of aminoglycosides to which a particular strain of enterococci might harbour resistance to synergy (1) streptomycin, (2) gentamicin, tobramycin and netilmicin and (3) kanamycin and amikacin (Eliopoulos and Moellering 1996). This has translated into clinical practice where high-level resistance to gentamicin and streptomycin is tested for as part of the management of enterococcal endocarditis. An important exception to this clustering of aminoglycosides and synergy is the absence of synergy of the penicillin–tobramycin combination in *Enterococcus faecium* due to the presence of a natural 6′-acetyltransferase in this species (Costa et al. 1993). By contrast, synergy is detectable even when strains of enterococci develop high-level resistance to penicillin and ampicillin through the acquisition of altered penicillin-binding proteins, although the concentrations of penicillin required to achieve synergy are high (Torres et al. 1993).

Staphylococci

Synergy has been shown in vitro for most strains of *Staphylococcus aureus* when β-lactams are combined with aminoglycosides (Eliopoulos and Moellering 1996). It has been suggested that the mechanism of synergy may involve mechanisms other than those seen with enterococci, and that β-lactams may suppress regrowth (selection of resistant mutants as is seen with *P. aeruginosa*) seen in time-kill experiments when aminoglycosides are used alone (Eliopoulos and Eliopoulous 1988).

In Vitro Pharmacodynamic Models

Comparatively, little work has been conducted on synergy in *S. aureus* using in vitro pharmacodynamic models.

Viridans Streptococci

There has been no work conducted on antimicrobial combinations for viridans streptococci with in vitro pharmacodynamic models.

Enterococci

Schwank and Blaser (1996) examined the effects of two penicillins and vancomycin combined with once- or thrice-daily netilmicin in an in vitro pharmacodynamic model and showed no difference between the two dosing schedules in terms of net killing at 48 h. Findings were the same for penicillins and vancomycin. This set the stage for clinical studies with once-daily aminoglycosides, instead of the conventional thrice-daily divided dosing. Similar findings for *E. faecalis* were shown by Houlihan et al. (2000) as demonstrated in Fig. 8.3. No difference in killing was detected whether the gentamicin was given once- or thrice-daily. Of note was that by 72 h of dosing, ampicillin alone was as effective as its combinations with gentamicin, despite the early more rapid kill observed with ampicillin plus gentamicin. With vancomycin plus gentamicin, synergy was much more pronounced, where either drug alone results in no killing over 72 h, and the combinations resulted in 4 $\log_{10}$ kill.

Staphylococci

In 1994, the Rybak group of investigators introduced a new in vitro pharmacodynamic model to study endocarditis by introducing infected fibrin clots into the apparatus (McGrath et al. 1994), which they subsequently named “simulated endocardial

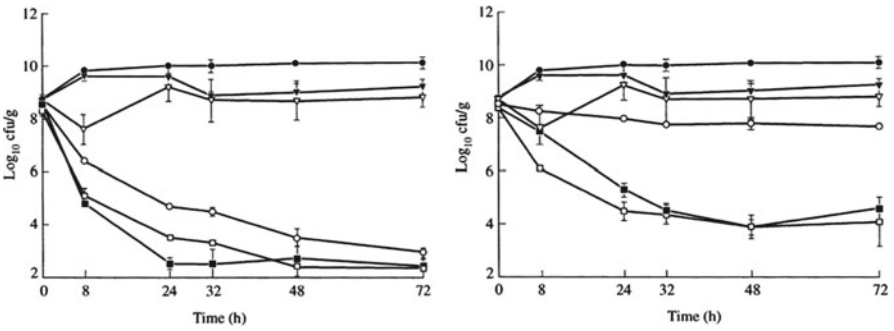

Fig. 8.3 Killing of *Enterococcus faecalis* in an in vitro pharmacodynamic model with combinations of ampicillin and vancomycin with gentamicin (from Houlihan et al. 2000). *Left panel*: *filled circle* growth control, *filled inverted triangle* gentamicin q8h, *open inverted triangle* gentamicin q24h, *open circle* ampicillin q6h, *filled square* ampicillin q6h + gentamicin q8h, *open square* ampicillin q6h + gentamicin q24h. *Right panel*: *filled circle* growth control, *filled inverted triangle* gentamicin q8h, *open inverted triangle* gentamicin q24h, *open circle* vancomycin q12h, *filled square* vancomycin q12h + gentamicin q8h, *open square* vancomycin q12h + gentamicin q24h

vegetations" (SEVs). They examined the effect of combining gentamicin with vancomycin and teicoplanin and showed significant enhancement of killing of *S. aureus* inside the fibrin clots compared to the glycopeptides alone. These findings were extended to rifampin when added to vancomycin in the same model, showing significant enhancement of killing compared to vancomycin alone, but not enhanced killing with added levofloxacin (Palmer and Rybak 1996). Subsequent studies, again with the same model have shown that (1) at high inocula, gentamicin can enhance the activity of nafcillin and linezolid, as can vancomycin to a lesser extent, but daptomycin cannot, although it is highly cidal itself in the model (LaPlante and Rybak 2004; Rose et al. 2008) and (2) when gentamicin exposure is shortened to the first 24 h, enhancement of vancomycin but not daptomycin can be achieved with a single high dose (5 mg/kg) (Tsuji and Rybak 2005); daptomycin killing is slowed by the addition of rifampin, but the effect compared to daptomycin is the same by 72 h (Rose et al. 2008; LaPlante and Woodmansee 2009).

Interest in combinations for methicillin-resistant strains has naturally attracted greater attention than for methicillin-susceptible strains. The earlier work from the Rybak group (Palmer and Rybak 1996; LaPlante and Rybak 2004; Tsuji and Rybak 2005) showed essentially no difference between the resistant and susceptible strains that they studied. Lee et al. employed the SEV model to show that vancomycin killing was enhanced by arbekacin and gentamicin whether or not the strain was susceptible to gentamicin (Lee et al. 2003), although the killing at 72 h was not greater than that with arbekacin alone. Recently, the novel combination of trimethoprim–sulfamethoxazole with daptomycin has been shown to synergistic with daptomycin in the SEV model against strains of *S. aureus* with reduced susceptibility to daptomycin (Steed et al. 2010).

Animal Model Data

Some of the most important work in achieving an understanding of the pharmacodynamics applicable to endocarditis treatment has been conducted in animal models. The state of the art in knowledge of pharmacodynamics in animal models of endocarditis 1992 was summarised in two reviews by Fantin and Carbon (1992) and Cremieux and Carbon (1992). Although the in vitro pharmacodynamic SEV model has been shown to be equivalent to the rabbit model, at least for some agents (Hershberger et al. 2000), animal models have continued to play an important role in determining the potential value of combination regimens in endocarditis (Le and Bayer 2003).

Caveats similar to those expressed for *P. aeruginosa* above apply to the animal model studies in this section. In many cases in the earlier studies, dosing schedules used in the animals would not necessarily have resulted in antimicrobial exposures similar to those of humans.

One important milestone in our understanding of pharmacokinetics/pharmacodynamics was to demonstration of the characteristics of antimicrobial penetration into endocardial lesions using autoradiography (Cremieux and Carbon 1992; Cremieux et al. 1989). This method overcame problems with interpretation experienced with previous attempt to determine penetration, especially the problem of homogenisation of lesions. Three diffusion patterns are now described (1) concentration at the periphery of vegetations with little or no penetration to the core (teicoplanin); (2) a concentration gradient between the periphery and the core of vegetations (ceftriaxone, penicillin and dalfopristin) and (3) homogenous diffusion throughout the vegetation (tobramycin, several fluoroquinolones, quinupristin and daptomycin) (Cremieux and Carbon 1992; Cremieux et al. 1992; Fantin et al. 1994).

Given the special environment of endocarditis vegetations, namely organisms in a low metabolic state and no neutrophils present, plus the varying characteristics of antimicrobial penetration into vegetation, the question arises as to whether the standard pharmacodynamic parameters (time above MIC, peak:MIC ratio and AUC:MIC ratio) and their know target values apply. In turn, if there were significant differences for endocarditis, what impact that might have on combination treatments?

The first study to examine this question called in question these concepts (Gengo et al. 1984). Using the rabbit model of *S. aureus* endocarditis, Gengo and colleagues administered approximately the same total daily dose of methicillin using four different dosing regimens and showed better survival at 14 days, after 5 days treatment, with 4- and 6-hourly regimens than 12-hourly or continuous infusion regimens. This suggested that time above MIC was not the most relevant pharmacodynamic parameter determining efficacy in endocarditis. The problem that this study threw up may well have been that of providing a sufficient antimicrobial gradient from plasma to vegetation, coupled with sufficient time above MIC within the core of the lesions. This would explain the poor efficacy of the least frequent dosing interval (high gradient but insufficient time above MIC in the lesions) and continuous infusion (very low gradient from plasma to outside of the lesion for a drug from

a class known to form a gradient itself from the periphery to the core of the lesion. This explanation is partially supported by the findings in the same rabbit model (albeit using *Escherichia coli*) of 3–4 day treatments with ceftriaxone and cefmenoxime (Pangon et al. 1987). In these studies, there was an inverse relationship between bacterial killing in vegetations and ceftriaxone trough levels, and the shorter half-life cefmenoxime was more effective at killing bacteria in lesions when given more frequently. In addition, a study of imipenem against an animal model of *P. aeruginosa* endocarditis showed greater killing in lesions when administered more frequently (Ingerman et al. 1986).

Of interest also is the summary of data accumulated on the efficacy of fluoroquinolones in experimental animal endocarditis, even though fluoroquinolones currently have no significant role in endocarditis treatment (Andes and Craig 1998). Andes and Craig have analysed data from 15 studies of a range of fluoroquinolones against viridans streptococci, methicillin-susceptible and -resistant *S. aureus* and Gram-negative bacteria and showed that the best correlation with reduction in mean colony-forming units per vegetation at 3–6 days of treatment was AUC_{24}/MIC. As their analysis used linear regression rather than the now more usual sigmoid exposure–response model, it was not possible to show clearly whether the AUC_{24}/MICs targets appropriate to Gram-positive bacteria (~30) and Gram-negatives (~100) in mouse thigh and pneumonia models applied also to the endocarditis model.

One of the problems with some of the animal models of endocarditis studies is the failure to adjust for the different kinetics in animals (usually rabbits or rats) compared to humans. For instance, in two studies which examined the effect of once-, twice- and/or versus thrice-daily doses of gentamicin in combination with penicillin ± ceftriaxone? used the same gentamicin doses that would be used in humans (3 mg/kg daily, 1.5 mg/kg twice daily and 1 mg/kg thrice daily) despite the more rapid clearance of gentamicin in rabbits (Gavaldà et al. 1995; Brandt et al. 1996). This translates to lower exposures than would be seen in humans given the same mg/kg dose. Similar issues arise with some antimicrobials used in many animal model studies of endocarditis.

An issue that has been attempted to be clarified by animal model studies is that of aminoglycoside regimen in combination with cell-wall active agents. This followed naturally from the studies of once-daily dosing of aminoglycosides for sepsis, where multiple clinical studies demonstrated that once-daily dosing was equivalent to thrice- or twice-daily divided dosing. For endocarditis though, it was unclear whether once-daily dosing would apply, given that the objective of the aminoglycoside combination was to take advantage of any synergy between the two drugs, which might be lost if the aminoglycoside was effectively absent from plasma for long periods of time in a dosing interval, as is usually seen with once-daily dosing.

The results of the animal model studies of endocarditis treated with combinations are listed in Table 8.4 and are summarised here:

Table 8.4 Animal model studies of endocarditis examining combination therapy

Reference	Animal (valve)	Organism(s)	Combination(s) examined	Humanised dosing?	Primary finding(s)
Vicente et al. (1981)	Rabbit (aortic)	*S. sanguis*	±Penicillin ± cefoxitin ±Penicillin + fosfomycin ±Cefoxitin ± fosfomycin	No	• All three combinations superior to single agents
Gavaldà et al. (1995)	Rabbit (aortic)	4 viridans streptococci with differing penicillin susceptibilities	Penicillin ± gentamicin	No	• Significant enhancement with either once-daily or thrice daily gentamicin
Brandt et al. (1996)	Rabbit (aortic)	*S. sanguis*	Penicillin ± gentamicin Ceftriaxone ± gentamicin	No	• Significant enhancement by both β-lactams with either once-daily, twice daily or thrice daily gentamicin • More frequent dosing (8- or 12-hourly) gave greater killing than once-daily
Bouvet et al. (1985)	Rabbit (aortic)	Nutritionally variant streptococcus	Penicillin ± gentamicin Penicillin ± amikacin Vancomycin ± gentamicin Vancomycin ± amikacin	No	• Gentamicin and amikacin enhanced penicillin but not vancomycin
Fass and Wright (1984)	Rabbit (aortic)	*E. faecalis*	Ampicillin ± gentamicin Mezlocillin ± gentamicin	No	• Gentamicin enhanced bacterial killing of ampicillin but not mezlocillin, which was quite effective alone
Fantin and Carbon (1990)	Rabbit (aortic)	*E. faecalis*	Penicillin ± netilmicin (3 netilmicin regimens)	Yes	• Netilmicin exposures similar to those in humans • Modest enhancement of penicillin by netilmicin • Higher divided doses (8-hourly) more effective than lower divided doses and once-daily higher dosing
Gavaldà et al. (1997)	Rabbit (aortic)	*E. faecalis*	Ampicillin ± gentamicin (2 once-daily gentamicin regimens)	Yes	• Small amount of additional killing with either gentamicin regimen compared to ampicillin alone

(continued)

Table 8.4 (continued)

Reference	Animal (valve)	Organism(s)	Combination(s) examined	Humanised dosing?	Primary finding(s)
Marangos et al. (1997)	Rabbit (aortic)	*E. faecalis*	Penicillin ± gentamicin (gentamicin once- or thrice-daily)	Yes	• Small amount of additional killing with either gentamicin regimen compared to penicillin alone
Join-Lambert et al. (1998)	Rabbit (aortic)	*E. faecalis*	Amoxycillin ± cefotaxime Amoxycillin ± gentamicin	Partly	• Small benefit from gentamicin combination but not from cefotaxime combinations
Rice et al. (1992)	Rat (aortic)	*E. faecalis*—high-level gentamicin resistant	Daptomycin ± fosfomycin	Yes	• No demonstrable benefit from adding fosfomycin to daptomycin
Caron et al. (1991)	Rabbit (aortic)	*E. faecium*—vancomycin and penicillin resistant	±Penicillin ± vancomycin ± gentamicin (all combinations)	Partly	• Best combinations were low-dose penicillin (low-dose) + vancomycin + gentamicin and penicillin(high-dose) + gentamicin • All other combination ineffective
Caron et al. (1992)	Rabbit (aortic)	*E. faecium*—vancomycin, teicoplanin and penicillin resistant	±Daptomycin ± gentamicin ±Teicoplanin ± gentamicin	Partly	• Gentamicin enhanced the effect of high-dose but not low-dose daptomycin • Smaller benefit of combining gentamicin with teicoplanin
Caron et al. (1993)	Rabbit (aortic)	*E. faecium*—vancomycin, teicoplanin and penicillin resistant	Penicillin ± vancomycin ± gentamicin Vancomycin ± gentamicin	Partly	• Only effective combination was penicillin(high-dose) + vancomycin + gentamicin
Vazquez et al. (1993)	Rabbit (aortic)	*E. faecalis* and *E. faecium*—ampicillin resistant	Sparfloxacin ± gentamicin Clinafloxacin ± gentamicin	Partly	• No enhancement of fluoroquinolone activity by gentamicin
Nicolau et al. (1996)	Rabbit (aortic)	*vanB E. faecalis*	±Vancomycin ± streptomycin ±Teicoplanin ± streptomycin	Yes	• Enhancement of both glycopeptides by gentamicin

Lefort et al. (1999)	Rabbit (aortic)	*vanB E. faecalis* (6 strains)	Vancomycin+gentamicin Teicoplanin+gentamicin	Yes	• Selection of gentamicin-resistant mutants universally with vancomycin combination and 50 % of time with teicoplanin combination if strain was teicoplanin resistant or hetero-resistant
Pérez Salmerón et al. (2006)	Rabbit (aortic)	*vanA E. faecium*	Quinupristin–dalfopristin ± gentamicin, teicoplanin, imipenem, levofloxacin	Yes	• No enhancement of quinupristin–dalfopristin by gentamicin or teicoplanin • Enhancement of quinupristin–dalfopristin by levofloxacin and imipenem
Sande and Johnson (1975)	Rabbit (aortic)	Penicillin-susceptible *S. aureus*	±Penicillin ± gentamicin ±Penicillin ± rifampin	No	• Enhancement (early only) of penicillin by gentamicin and rifampin
Sande and Courtney (1976)	Rabbit (aortic)	Methicillin-susceptible *S. aureus*	±Nafcillin ± gentamicin	No	• Gentamicin enhanced nafcillin compared to nafcillin alone
Miller et al. (1978)	Rabbit (aortic)	Methicillin-susceptible *S. aureus*	±Nafcillin ± gentamicin	Partly	• Resistant subpopulation emerged on gentamicin alone (small colony variants) • Nafcillin in combination more effective than either alone at day 4 but not day 6 of therapy • Nafcillin controlled the emergence of gentamicin-resistant subpopulation
Kaatz et al. (1989)	Rabbit (aortic)	Methicillin-susceptible *S. aureus* (2 strains)	Ciprofloxacin ± rifampin	Partly	• Enhancement of ciprofloxacin by rifampin in 1 of 2 strains only
Chambers and Miller (1987)	Rabbit (aortic)	Methicillin-resistant *S. aureus*	Cephalothin ± gentamicin	Yes	• Enhancement of (weak) cephalothin by gentamicin • Prevention of emergence of resistance to gentamicin but not cephalothin

(continued)

Table 8.4 (continued)

Reference	Animal (valve)	Organism(s)	Combination(s) examined	Humanised dosing?	Primary finding(s)
Rodríguez et al. (1987)	Rabbit (aortic)	Methicillin-resistant *S. aureus*	Vancomycin ± gentamicin Fosfomycin ± gentamicin	No	• Gentamicin enhanced the effect of vancomycin and fosfomycin
Fantin et al. (1993)	Rabbit (aortic)	Methicillin-resistant *S. aureus*	±Vancomycin ± fusidic acid	Partly	• No enhancement of vancomycin by fusidic acid • Pronounced inoculum effect with fusidic acid alone
Chambers et al. (1995)	Rabbit (aortic)	Methicillin-resistant *S. aureus*	±Ampicillin–sulbactam ± rifampin	No	• Rifampin enhanced the effect of ampicillin–sulbactam • Both drugs alone ineffective • Rifampin-resistant mutants emerged on rifampin alone • Combination greatly reduced rate of rifampin resistance emergence
Batard et al. (2002)	Rabbit (aortic)	Methicillin-resistant *S. aureus* (2 strains: 1 MLS_B-S, and 1 MLS_B-R)	±Quinupristin–dalfopristin ± gentamicin	Yes	• Quinupristin–dalfopristin more effective against MLS_B-S strain • Gentamicin did not enhance the effect of quinupristin–dalfopristin
Chiang and Climo (2003)	Rabbit (aortic)	Methicillin-resistant *S. aureus*	±Vancomycin ± linezolid	Partly	• Vancomycin alone superior to linezolid alone • Linezolid antagonised the vancomycin effect
Jacqueline et al. (2004)	Rabbit (aortic)	Methicillin-resistant *S. aureus* (2 strains)	±Linezolid ± gentamicin	Yes	• Small effect from linezolid alone, significant enhanced by combination with gentamicin
Tsaganos et al. (2008)	Rabbit (aortic)	Methicillin-resistant *S. aureus*	±Linezolid ± rifampin ±Vancomycin ± rifampin	Yes	• Rifampin enhanced the activity of both linezolid and vancomycin
Fox et al. (2006)	Rabbit (aortic)	Vancomycin-resistant *S. aureus*	±Nafcillin ± vancomycin	Yes	• Combination was effective compared to either agent alone

Viridans Streptococci

1. Combinations that appear synergistic for susceptible strains
   (a) Penicillin plus gentamicin (Gavaldà et al. 1995; Brandt et al. 1996; Vicente et al. 1981; Bouvet et al. 1985)
   (b) Penicillin plus amikacin (Bouvet et al. 1985)
   (c) Penicillin plus fosfomycin (Vicente et al. 1981)
   (d) Ceftriaxone plus gentamicin (Brandt et al. 1996)
2. Combinations with no apparent synergy for susceptible strains
   (a) Vancomycin plus either gentamicin or amikacin (Bouvet et al. 1985)
3. Conflicting data on the superiority of once- versus thrice-daily dosing of gentamicin (Gavaldà et al. 1995; Brandt et al. 1996)
4. Enhancement of penicillin efficacy by gentamicin across a range of penicillin MICs (Gavaldà et al. 1995)

Enterococci

1. Combinations that appear synergistic for susceptible strains
   (a) Ampicillin plus gentamicin (Fass and Wright 1984; Gavaldà et al. 1997)
   (b) Amoxicillin plus gentamicin (Join-Lambert et al. 1998)
   (c) Penicillin plus netilmicin (Fantin and Carbon 1990)
   (d) Penicillin plus gentamicin (Marangos et al. 1997)
2. Combinations with no apparent synergy (high-level gentamicin-resistant strain)
   (a) Daptomycin plus fosfomycin (Rice et al. 1992)
3. Combinations with apparent synergy against penicillin- and vancomycin-resistant strains
   (a) Penicillin plus vancomycin plus gentamicin (Caron et al. 1991, 1993)
   (b) High-dose penicillin plus gentamicin (Caron et al. 1991)
   (c) High-dose daptomycin plus gentamicin (Caron et al. 1992)
   (d) Vancomycin plus with streptomycin (Nicolau et al. 1996) or gentamicin (Lefort et al. 1999) (*vanB* strains)
   (e) Teicoplanin plus either gentamicin (Caron et al. 1992; Lefort et al. 1999) or streptomycin (Nicolau et al. 1996)
4. Combinations with no apparent synergy for susceptible strains
   (a) Sparfloxacin or clinafloxacin plus gentamicin (Vazquez et al. 1993)
5. Selection of gentamicin-resistant subpopulations in vancomycin-resistant strains with glycopeptide plus gentamicin combinations (Lefort et al. 1999)

Staphylococci

1. Combinations that appear to be synergistic for penicillin-susceptible strains
   (a) Penicillin plus gentamicin (Sande and Courtney 1976)
   (b) Penicillin plus rifampin (Sande and Courtney 1976)
2. Combinations that appear synergistic for methicillin-susceptible strains
   (a) Nafcillin plus gentamicin (but only early in therapy) (Sande and Courtney 1976; Miller et al. 1978)
3. Unpredictable apparent synergy (strain dependent)
   (a) Ciprofloxacin plus rifampin (Miller et al. 1978)
4. Combinations that appear synergistic for methicillin-resistant strains
   (a) Cephalothin plus gentamicin (Chambers and Miller 1987)
   (b) Vancomycin plus gentamicin (Rodríguez et al. 1987)
   (c) Fosfomycin plus gentamicin (Rodríguez et al. 1987)
   (d) Ampicillin-sulbactam plus rifampin (Chambers et al. 1995)
   (e) Vancomycin plus rifampin (Tsaganos et al. 2008)
   (f) Linezolid plus gentamicin (Jacqueline et al. 2004)
   (g) Linezolid plus rifampin (Tsaganos et al. 2008)
5. Combinations with no apparent synergy for methicillin-resistant strains
   (a) Vancomycin plus fusidic acid (Fantin et al. 1993)
   (b) Quinupristin-dalfopristin plus gentamicin (Batard et al. 2002)
   (c) Vancomycin plus linezolid (antagonism) (Chiang and Climo 2003)
6. Combinations that appear synergistic for vancomycin-resistant (*vanA*) strains
   (a) Vancomycin plus nafcillin (Fox et al. 2006)

The general conclusion from these studies is that combinations of β-lactams and aminoglycosides appear synergistic in animal models of endocarditis, provided that the organism is at least moderately susceptible to penicillins. Some potentially valuable combinations have not been studied in detail: β-lactams plus rifampin for instance, and few data exist from animal models are available to answer questions about the approach to enterococci with high-level aminoglycoside resistance. A distinct feature of these animal models of endocarditis is that almost all assessments are made between day 3 and day 6 of therapy, in contrast to the clinical situation where therapy last for 2 weeks (viridans streptococci) or 6 weeks (enterococci and staphylococci) usually.

Clinical Studies

High-quality evidence for the benefit of combination therapy over single agent therapy in Gram-positive endocarditis is lacking. Indeed, in the absence of high-quality

evidence, many of the current recommendations for the management of Gram-positive endocarditis are necessarily based on in vitro and animal model findings only, combined with largely uncontrolled studies anecdotal reports and cumulative clinical experience (Baddour et al. 2005).

Viridans Streptococci

Two retrospective and one prospective study have examined the potential benefit of combination therapy for streptococcal (non-enterococcal) endocarditis. Malacoff et al. reviewed 64 patients with streptococcal endocarditis and noted two relapses in 46 patients who received monotherapy compared to 2 or 18 patients who received combination therapy (mostly penicillin plus either streptomycin or gentamicin) (Malacoff et al. 1979). A similar experience was documented by Tuazon et al. in 1986 in the same form of endocarditis (Tuazon et al. 1986). These authors noted one relapse in 22 monotherapy patients versus none in 26 combination therapy patients. In both of these studies, there were wide variations in duration of therapy and dosing regimens.

The only prospective study of streptococcal endocarditis was one comparing ceftriaxone 2 g once daily for 4 weeks with ceftriaxone 2 g once-daily plus 3 mg/kg of gentamicin once daily for 2 weeks (Sexton et al. 1998). One of the main objectives of the study was to demonstrate that an effective 2-week regimen suitable for outpatient treatment could be devised. In this, the study was successful. Clinical treatment failure was observed once in each arm: monotherapy $n=26$, combination therapy $n=25$, while only one microbiological failure was observed, and that was in the combination therapy arm.

Enterococci

There are no randomised controlled trial data confirming the superiority of combination therapy for enterococcal endocarditis, generally an aminoglycoside with a cell-wall active agent. One retrospective analysis was conducted at a time when the doses of penicillin were sometimes less than what would be considered adequate by today's standard (Geraci and Martin 1954). Instead, as summarised by Megran in 1992, the case for the superiority combination therapy has been built on the cumulative evidence of in vitro and animal model data, to which has been added extensive clinical experience, both published and unpublished (Megran 1992). In the combination of a cell-wall active agent, the aminoglycoside is always given at low doses in order to minimise toxicity while taking advantage of potential synergy. This is considered particularly important as the current standard for enterococcal endocarditis is to administer combination therapy throughout the course of 4–6 week total (Baddour et al. 2005). Recently, evidence has been presented from Sweden that a shortened course of the aminoglycoside (about 2 weeks) does not compromise efficacy (Olaison and Schadewitz 2002).

Staphylococci

Combination therapy emerged as a possibility for *S. aureus* endocarditis in the mid-1970s after anecdotal reports of failures with β-lactam agents that were converted to cures by the addition of gentamicin (Murray et al. 1976). Three prospective and one retrospective study had examined single versus combination therapy for *S. aureus* endocarditis (Abrams et al. 1979; Korzeniowski and Sande 1982; Ribera et al. 1996; Drinković et al. 2003). In each of the prospective studies, the combination examined was that of a β-lactam plus gentamicin. In the retrospective study, other combinations were also included. The results observed in these studies are summarised in Table 8.5.

One of difficulties of interpreting the outcomes of these studies is the different outcomes between L-sided native valve, R-sided native valve and prosthetic valve endocarditis, in order of increasingly poorer prognosis. However, the results could be summarised by observing that the addition of aminoglycoside appears to offer no benefit in the first two varieties of staphylococcal endocarditis, but may be an important part of regimens used to treat prosthetic valve endocarditis. It is interesting to note that the current US recommendations list the gentamicin combination still as optional for native valve endocarditis (Baddour et al. 2005), although only for the first 3–5 days. However, the toxicity issue with the use of concomitant gentamicin even on this schedule has recently been re-addressed (Cosgrove et al. 2009). Reviewing results from a recent large multicentre study comparing daptomycin with combination cell-wall active agent plus gentamicin found 22 % nephrotoxicity in patients treated with the combination therapy compared to only 8 % in patients receiving vancomycin. As a corollary, a recent retrospective analysis of 87 patients with *S. aureus* endocarditis or prolonged bacteremia who did or did not receive additional aminoglycoside therapy has shown, using multivariable analysis a significantly lower rate of relapse/recurrence of bacteremia: odds ratio 0.26 (CI 0.07–0.98) (Lemonovich et al. 2011).

Conclusions

Combination therapy is widely used in the management of serious *P. aeruginosa* infection and Gram-positive endocarditis. Despite extensive in vitro static, pharmacodynamic and animal model studies, uncertainties persist about the utility of combinations, especially given the potential for toxicity associated with concomitant aminoglycoside use. Ultimately, an in vitro or animal model of the pharmacodynamic interaction of drug classes that can be shown to predict clinical outcomes is still required. Such a model does not currently exist.

Table 8.5 Clinical studies of *S. aureus* endocarditis in adults examining combination therapy

Reference {patient group}	Site of infection	Definitive therapy	Findings
Abrams et al. (1979) {IV drug users}	Left-sided $n=2$ Right-sided $n=20$ Both $n=3$	Oxacillin or penicillin or cephalothin ± gentamicin 80 mg (~1 mg/kg) 8-hourly for the first 2 weeks	• No differences in time to defervescence • In either group: no bacteriological failures, no relapses, no surgery, no mortality
Korzeniowski and Sande (1982) {native valve infection}	Left-sided $n=29$ Right-sided $n=33$ Both $n=16$	Nafcillin ± gentamicin 1 mg/kg 8-hourly for the first 2 weeks	• Relapse: nafcillin 1/35, nafcillin + gentamicin 1/43 • Death: nafcillin 2/35, nafcillin + gentamicin 6/43 • Surgery: nafcillin 3/35, nafcillin + gentamicin 2/43 • R-sided disease: more rapid defervescence and more rapid clearance of bacteremia • L-sided disease: more rapid clearance of bacteremia but higher rate of renal impairment
Ribera et al. (1996) {IV drug users}	R-sided only $n=74$	Cloxacillin for 14 days ±gentamicin 1 mg/kg 8-hourly for the first 7 days	• Relapse: cloxacillin 0/38 • Death: cloxacillin 1/38, cloxacillin + gentamicin 2/36 • Active infection at 14 days: cloxacillin 3/38, cloxacillin + gentamicin 1/36
Drinković et al. (2003) {patients requiring valvular surgery; includes patients with coagulase-negative staphylococcal infection}	Native valve $n=74$ Prosthetic valve $n=29$ L-sided $n=65$ R-sided $n=6$ Both $n=3$	Flucloxacillin or penicillin or vancomycin (mostly) ±gentamicin 1 mg/kg 8-hourly, ±rifampin in some patients	• Native valve +ve cultures at the time of surgery: monotherapy 19/32, combination 23/42 • Prosthetic valve +ve cultures at time of surgery: monotherapy 8/14, combination 14/27 (significantly lower when adjusted for duration of therapy)

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Part II
Clinically Oriented Chapters

Chapter 9
Aminoglycosides

Catharine C. Bulik, Charles H. Nightingale, and David P. Nicolau

Abstract Aminoglycoside antibiotics, bactericidal agents which irreversibly bind to the 30s ribosomal subunit and cause the inhibition of protein synthesis, have remained a vital aspect of the antimicrobial armamentarium since their introduction over 5 decades ago. Aminoglycosides are broad-spectrum agents with activity against a wide range of aerobic Gram-negative and Gram-positive pathogens, in addition to certain mycobacteria. Due to poor oral absorption, aminoglycosides are mainly administered as parenteral or inhalational agents. They demonstrate low protein binding and distribute freely into the interstitial or extracellular fluid. These agents are eliminated from the body via glomerular filtration with 99% of a dose excreted unchanged in the urine. As such, the aminoglycosides require dose adjustment in the presence of renal dysfunction to avoid drug related toxicities. Understanding of the pharmacokinetic-pharmacodynamic properties of the aminoglycosides has allowed practitioners to utilize these agents with minimization of drug related toxicities. Pharmacodynamically optimized dosing of the aminoglycosides includes the administration of large doses once daily to both minimize the drug toxicities associated with elevated trough levels, and to optimize the post-antibiotic effects observed with these agents. For patients who are not candidates for

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high-dose once daily dosing, these patients are administered aminoglycosides via the "traditional method" which involves multiple administrations of smaller doses every 8 hours. Overall, despite some drug related toxicities which can be overcome through the optimization of PK-PD, the aminoglycosides remain a vital component of a practitioner's arsenal against infection.

Keywords Aminoglycosides • Pharmacokinetics • Pharmacodynamics • Once-daily dosing

Introduction

Aminoglycoside antibiotics have remained an important player in the antimicrobial arsenal since their introduction over five decades ago. Despite the extensive experience with these agents, the major obstacle with their use is the potential for drug-related toxicity. However, through the application of pharmacodynamics, dosing strategies can be employed which lead to improved efficacy and diminished toxicodynamics. As a result of the understanding of these principles, parenteral dosing techniques for the aminoglycosides have been modified from the administration of frequent small intermittent dosages to once-daily regimens which not only optimizes the pharmacodynamic and toxicodynamic profiles but also substantially reduces costs. The combination of these principles with the in vitro activity, proven clinical effectiveness, and synergistic potential of the aminoglycosides are the rationale behind their continued use in the management of serious infections.

Mechanism of Action of Aminoglycosides

The bactericidal activity of the aminoglycosides is thought to be partially ribosomally mediated. The intact bacterial ribosome is a 70S particle that consists of two subunits (50S and 30S) and it is the smaller 30S ribosomal submit, which contains the 16S rRNA that is the primary target for aminoglycosides. By irreversibly binding to this subunit the aminoglycoside interferes with the reading of the genetic code, leading to inhibition of protein biosythesis (Davies 1983). Nonetheless, the inhibition of protein synthesis is not thought to be the exact mechanism of bactericidal activity. Other agents whose sole mechanism of action is inhibition of protein synthesis are considered bacteriostatic agents, which suggest suggests additional unknown mechanisms of activity that lend to the bactericidal activity of aminoglycosides.

However, in order to reach their ribosomal target, aminoglycosides must initially cross the outer membrane and/or the cytoplasmic membrane of the organism. The initial step involves ionic binding of the charged aminoglycoside molecule to the cell surface and is followed by energy- and oxygen-dependent transport mechanisms (Edson and Terrell 1999). The rapid initial binding of the aminoglycoside to

the cell membrane accounts for the rapid bactericidal activity which increases with increasing aminoglycoside concentration. It is this characteristic that accounts for the aminoglycosides' concentration- or dose-dependent killing and is one of the major rationales for dosing aminoglycoside on a once-daily basis in order to maximize bacterial killing.

Microbiologic Spectrum

The aminoglycosides are broad-spectrum antibiotics with activity against a wide range of aerobic Gram-negative and Gram-positive pathogens in addition to certain mycobacteria. However, it is their in vitro activity against Gram-negative pathogens that is most notable. These pathogens include common clinical isolates of *Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Serratia marcescens*, *Proteus* spp., *Providencia* spp., *Morganella* spp., and *Pseudomonas aeruginosa*. Overall the aminoglycosides are considered active against these pathogens, but individually substantial differences in antimicrobial potency exist among the various aminoglycosides. For example, even though the antimicrobial spectra of gentamicin and tobramycin are quite similar, tobramycin is generally more active in vitro against *P. aeruginosa*, whereas gentamicin is more active against *S. marcescens*.

Aminoglycosides are generally active against *Staphylococci* spp. yet alone do not inhibit *Enterococci* spp. and *Streptococci* spp. As a consequence, aminoglycosides are not generally advocated as single agents for infections due to Gram-positive pathogens but are frequently administered in combination with a cell wall active agent to provide synergy in the treatment of serious infections due to *Staphylococci* spp., *Enterococci* spp., and *Viridans streptococci*.

Pharmacokinetic Characterization

Absorption

Due to a highly charged nature, aminoglycosides demonstrate both a poor oral and poor gastrointestinal absorption. This highly charged nature leads to increased hydrophilicity and poor membrane permeability and also causes these agents to poorly penetrate intact skin. However, detectable blood levels have been found when used as a topical antibacterial for large areas of denuded skin (i.e., thermal injury). Due to their ability to penetrate extremely well into body spaces with large serosal surfaces, the use of aminoglycosides for local irrigation of closed body cavities may result in considerable systemic accumulation and potential toxicity (Edson and Terrell 1999).

Consistent with the concerns for potential systemic toxicity from their topical usage, the swallowed portions of inhaled or nebulized aminoglycosides may cause detectable aminoglycoside serum concentrations and the development of toxicity in patients with poor renal function.

As a consequence of poor oral bioavailability the aminoglycosides must be given parenterally in order to achieve adequate systemic serum concentrations. While the intramuscular route is well tolerated and results in essentially complete absorption, intravenous administration is generally preferred because of the rapid and predictable serum profile.

Distribution

The aminoglycosides demonstrate relatively low protein binding (approximately 10 %) and therefore freely distribute into the interstitial or extracellular fluid. The apparent volume of distribution of this class of agents is approximately 25 % of the total body weight, which corresponds to the estimated extracellular fluid volume. In patients in whom there is an absence of disease or infection the volume of distribution can be approximated at 0.25–0.3 L/kg. However, in edematous states, such as ascites and burns, the volume of distribution increases. In patients who are obese, pregnant, or in the intensive care unit, there are substantial alterations in the volume of distribution which require dosage and/or schedule modifications to maintain the desired serum profile.

In general, due to the low protein binding of aminoglycosides, they are able to distribute well into extravascular space. However, due to the large size of the molecules, their low lipid solubility, and their highly charged nature, the aminoglycosides penetrate poorly into human cells and therefore the concentrations attained in tissue and body fluids are less than that obtained in serum. The exception to this is in specialized cells such as the tubular cells of the renal cortex and kidney and in the perilymph of the inner ear which have active transport mechanisms for aminoglycosides. In these tissues, the concentrations of aminoglycosides exceed that of the plasma. Penetration of aminoglycosides into cerebral spinal fluid and vitreous fluid is poor regardless of the state of inflammation therefore direct instillation is often required to provide sufficient concentrations at these sites. Additionally, as these agents cross the placenta, the potential risk to the fetus and mother must be considered prior to use.

Elimination

The kidneys, via glomerular filtration, are responsible for essentially all aminoglycoside elimination from the body with 99 % excreted unchanged in the urine. As a result, there is a proportional relationship between drug clearance and glomerular

filtration rate, which is routinely utilized to assist with aminoglycoside dosage modification (Zarowitz et al. 1992). In adults and children older than 6 months with normal renal function the elimination half-life is approximately 2–3 h. In patients with renal dysfunction, there is a considerable elongation of the aminoglycoside half-life. Due to the maturation of the glomerular filtration rate, for premature infants, infants with low-birth weight, and those less than 1 week old the half-life is 8–12 h, whereas the half-life decreases to 5 h for neonates whose birth weight exceeds 2 kg (Rhodin et al. 2009). The aminoglycosides undergo active reabsorption in the proximal renal tubular cells and following the elimination phase, there is a slow terminal elimination phase (30–700 h) due to the release of the aminoglycoside from these cells back into the urine.

Pharmacodynamic Overview

Pharmacodynamics can be defined as the relationship between the changing concentrations observed with drug dosing and the killing of the bacteria (Turnidge 2003). Each drug has a distinct pharmacodynamic profile due to the influence of drug concentration on the rate and extent of bactericidal activity. The pharmacodynamic profile of the aminoglycosides has been characterized extensively both in vitro and in vivo. Based on these data the aminoglycosides have a pharmacodynamic profile that is in line with agents in which the rate and extent of bactericidal activity is dependent upon drug concentration (e.g. fluoroquinolones and aminoglycosides) rather than agents such as the β-lactams which have bactericidal activity independent of drug concentration when their concentration exceeds a low multiple of the minimum inhibitory concentration (MIC) (Begg et al. 1992; Craig and Ebert 1990; Drusano et al. 1993; Dudley 1991).

These principles were demonstrated in an experiment conducted by Craig and Ebert (1990) in which bacteria were exposed to various multiples of the MIC of ciprofloxacin, tobramycin, and ticarcillin. Ticarcillin exhibited dose-independent bactericidal activity as evident by the lack of difference in the rate of bactericidal activity that was noted when the concentration exceeded four times the MIC value of the isolate.

In contrast, with the same multiples of the MIC value, tobramycin and ciprofloxacin demonstrated concentration-dependent killing evident by the number of organisms decreasing more rapidly with each rising MIC interval. Based on in vitro data, optimum bactericidal activity for the aminoglycosides is achieved when the exposure concentration is approximately 8–10 times the MIC value (Davis 1987; Ebert and Craig 1990). In addition to maximal bactericidal activity, Blaser and colleagues have demonstrated that a peak/MIC ratio of 8:1 was correlated with a decrease in the selection and regrowth of resistant subpopulations that occur during treatment with netilmicin (Blaser et al. 1987).

For all antimicrobials, drug concentrations at the site of action (i.e., the ribosome for the aminoglycosides) would be the optimal measure to determine activity. Since

we cannot yet access this concentration, various pharmacokinetic parameters such as AUC (area under the concentration–time curve), maximum observed concentration (C_{max} or peak), and half-life are often assessed in correlation with the MIC value of the pathogen to produce pharmacodynamic parameters such as the AUC/MIC ratio, peak/MIC ratio parameters, and the time which the drug concentration remains above the MIC (time > MIC) to describe drug efficacy. For the aminoglycosides the AUC/MIC ratio, peak/MIC ratio, and time > MIC have all been shown to be associated with efficacy (Blaser et al. 1987; Leggett et al. 1991). However, since the aminoglycosides demonstrate concentration-dependent killing and a relatively long postantibiotic effect, the influence of the time > MIC is not as important when compared to the influence of the peak concentration. As a result the pharmacodynamic parameter which is believed to best characterize the profile of the aminoglycosides in vivo is the peak/MIC ratio.

The discovery of the peak/MIC ratio as being the best pharmacodynamic parameter, and therefore associated with treatment success, for the aminoglycosides has been demonstrated in many studies in man. Moore and colleagues demonstrated that higher peak concentrations were associated with improved treatment outcomes in both Gram-negative pneumonia and Gram-positive bacteremia (Moore et al. 1984a, b). In a study designed to evaluate the relationship between the peak/MIC ratio and clinical outcomes, Moore and colleagues further proved the importance of the peak/MIC ratio for aminoglycosides (Moore et al. 1987). This study looked at data collected from four randomized, double-blind, controlled clinical trials which utilized gentamicin, tobramycin, or amikacin for the treatment of Gram-negative bacterial infections (Deziel-Evans et al. 1986). Peak concentration (C_{max}) was defined as the highest concentration determined during therapy, while the mean peak concentration was calculated as the average of all C_{max} values during the course of treatment. The investigators demonstrated that high maximal and mean peak aminoglycoside concentration (8.5 ± 5.0 μg/ml and 6.6 ± 3.9 μg/ml, respectively) to MIC ratios were significantly ($P < 0.00001$ and $P < 0.0001$, respectively) correlated with clinical response. Of the 188 patients who had a clinical response to therapy, the C_{max}/MIC ratio average value was 8.5 ± 5.0 μg/ml, whereas the 48 nonresponders had a ratio of 5.5 ± 4.6 μg/ml ($P < 0.00001$). In another study by Deziel-Evans and colleagues, it was demonstrated that a 91 % cure rate was observed in patients with peak/MIC ratios greater than 8, while only a 12.5 % cure rate was observed for patients with ratios less than or equal to ≤4 in a retrospective study with 45 patients (Deziel-Evans et al. 1986). Finally, in another study by Keating and coworkers, response rates of 57, 67, and 85 % were observed in neutropenic patients with mean serum aminoglycoside concentration/MIC ratios of 1–4, 4–10, and greater than 10, respectively (Keating et al. 1979). In addition to the above-noted studies, many others have also observed beneficial correlations between serum concentrations or pharmacodynamic parameters and therapeutic outcomes in patients treated with aminoglycosides (Anderson et al. 1976; Noone et al. 1974; Reymann et al. 1979).

More recently, Kashuba and coworkers reported that achieving an aminoglycoside peak/MIC ratio of ≥10 within 48 hours of initiation of therapy for Gram-negative pneumonia resulted in a 90 % probability of therapeutic response by day

7 of therapy (Kashuba et al. 1998, 1999). The authors also note that aggressive aminoglycoside dosing (initial dose of 7 mg/kg) followed by individualized pharmacokinetic monitoring should maximize the rate and extent of response in this patient population.

These trials in addition to many more have shown that the peak/MIC ratio is the pharmacodynamic parameter that is best associated with aminoglycoside efficacy. Furthermore, when this peak/MIC ratio is above a specific level of 8–10, patient outcomes are shown to improve. This pharmacodynamic parameter, in addition the availability of a long postantibiotic effect has lead to the thought that in order to optimize aminoglycoside dosing, these drugs would best serve to be dosed as a high-dose once daily.

Postantibiotic Effect

The postantibiotic effect (PAE) is defined as the persistent suppressive activity against bacterial growth after limited exposure of bacteria to an antibiotic. Aminoglycosides have been shown to demonstrate a clinically meaningful PAE with a range of 0.5–7.5 h reported in the literature (Zhanel et al. 1991). Several factors can influence the determination of the PAE in vitro including, but not limited to, the actual organism, concentration of antibiotic, duration of antimicrobial exposure, and the effect of antimicrobial combinations.

Unlike β-lactam antibiotics which demonstrate PAEs against only Gram-positive organisms, aminoglycosides exhibit a PAE against both Gram-positive and Gram-negative organisms (Bundtzen et al. 1981). However, the PAE duration differs depending on the bacteria. For example, the duration of the PAE following exposure of *P. aeruginosa* to gentamicin and tobramycin are 2.2 h and 2.1 h, respectively, while those of *E. coli* are 1.8 h and 1.2 h, respectively.

The concentration of the aminoglycoside also has a major effect on the PAE (Vogelman et al. 1988; Hessen et al. 1989; McGrath et al. 1993; Vogelman and Craig 1985). The maximum concentration of aminoglycoside required to exert the maximal PAE effect is difficult to determine due to the extensive bacterial kill at high drug concentrations. In contrast, the PAE of penicillin G gradually increases up to a point of maximal effect at a concentration 8–16 times the MIC (Vogelman and Craig 1985; Odenholt-Tornqvist 1989; Craig et al. 1998).

The duration of PAE can also vary according to which antibiotics are given concurrently. The combined effect of aminoglycosides and cell wall inhibitors on the duration of the PAE has been studied extensively (Dornbusch et al. 1989; Fursted 1988; Winstanley and Hastings 1989; Gudmundsson et al. 1991). In general, these combinations produced additive effects (e.g., similar to the sum of PAEs for the individual drugs) or synergistic effects (e.g., at least 1 h longer than the sum of PAEs for the individual drugs) in *S. aureus* and various *Streptococci spp*. When focusing on the effects of the combinations against Gram-negative bacilli, they were found to be mainly additive or indifferent (e.g., no different from the longest of the individual

PAEs). An exception to this is in the addition of tobramycin to rifampin which can achieve prolonged PAEs in Gram-negative bacilli and demonstrate PAE synergism against *P. aeruginosa*, *E. coli*, and *K. pneumonia* (Gudmundsson et al. 1991).

A major drawback to the clinical application of aminoglycoside PAEs determined in vitro is the lack of consideration of the host's immune system. Some efforts have been made to include host immunity by using other terminology such as postantibiotic leukocyte enhancement (PALE) and postantibiotic sub-MIC effect. PALE is a phenomenon in which pathogens in the PAE phase are more susceptible to the antimicrobial effect of human leukocytes than non-PAE controls. Postantibiotic sub-MIC effect illustrates the joining of the PAE and the additive effects of exposure to sub-MIC levels (Cars and Odenholt-Tornqvist 1993).

Despite the limitations involved in predicting the exact duration of PAE, it is an important factor to be considered when developing a drug regimen. The precise mechanisms of the PAE are largely unknown but several hypotheses have been suggested. These hypotheses include limited persistence of the antibiotic at the site of action, recovery from nonlethal damage to cell structures, the time required for synthesis of new proteins or enzymes before growth, and drug-induced nonlethal damage due to the irreversible binding of the aminoglycoside to the bacterial ribosomes (Vogelman and Craig 1985; Craig and Vogelman 1987).

Despite the numerous data available regarding in vitro PAE, there is less information in respect to the in vivo PAE. Animal models are often used to evaluate the in vivo PAE and based on these models, factors which have been found to affect the in vivo PAE include the infection site, type of organism, the drug dose, simulation of human pharmacokinetics, and the presence of leukocytes (Craig 1993). Animal models have shown that the PAE varies according to the site of infection with PAEs associated with *K. pneumoniae* in the mouse pneumonia model being roughly 1.5–2.5 times longer than that observed in the mouse thigh model at the corresponding dose.

Similar to the in vitro PAE, the in vivo PAE is also known to vary according to the type of organism. Again, similar to the in vitro PAEs the duration of the in vivo PAE of aminoglycosides are prolonged 1.9- to 2.7-fold by the presence of leukocytes (Craig 1993).

Regardless of the determination of PAE in vitro versus in vivo, this effect has a major impact on antimicrobial dosing. For antibiotics with a longer PAE, the dosing frequency may be less than that of antibiotics with a shorter PAE. Therefore, PAE may be one of the rationales for the implementation of once-daily aminoglycoside dosing.

Resistance and Synergy

Resistance to aminoglycosides is conferred by three mechanisms: impaired drug uptake, mutations of the ribosome, and enzymatic modification of the drug. Intrinsic resistance is often due to impaired uptake, while acquired resistance usually results from acquisition of transposon- and plasmid-encoded modifying enzymes (Mingeot-Leclercq et al. 1999). Pharmacodynamically optimized dosing strategies that

minimize these mechanisms of resistance include selecting dosing regimens which maximize the rate and extent of bacterial kill.

Adaptive resistance describes the refractoriness of an organism to the bactericidal activity of the aminoglycoside. This phenomenon has been demonstrated in vitro by exposing *P. aeruginosa* to concentrations of gentamicin below or at the MIC value of the organism (Daikos et al. 1990, 1991). Continuous exposure of the organism to the aminoglycoside allows the organisms which display adaptive resistance to survive increasing concentrations of gentamicin (Gilleland et al. 1989). Adaptive resistance has also been shown to occur in vivo in neutropenic mice with *P. aeruginosa* thigh infections treated with netilimicin. When dosing the netilimicin at 8 h intervals, significant bactericidal activity was seen, but when the dosing interval is decreased to 4 or 6 h, the bactericidal effect decreases and murine mortality increased (Gilleland et al. 1989). Based on this observation, longer dosing intervals, as can be achieved with the pharmacodynamically based once-daily aminoglycoside dosing approach which allows for a drug-free period in which the bacteria are not exposed to an aminoglycoside, should further preserve the antibacterial activity of these agents after multiple doses.

Aminoglycosides exhibit synergistic bactericidal activity when given in combination with cell wall active agents (Owens et al. 1997; Marangos et al. 1997). However, when combination therapy is advocated to achieve synergy for Gram-negative organisms, maximally effective doses of both agents should be maintained because synergy does not occur universally for all pathogens (Owens et al. 1997; Hallander et al. 1982).

Toxicodynamics

Aminoglycosides, like the majority of other antimicrobials, display a variety of adverse effects. The precise cellular mechanism of toxicities has not been determined, but theories exist that include aberrant vesicle fusion, mitochondrial toxicity/free radical generation, and decreased protein synthesis either by reduced transcription or translation after aminoglycoside exposure (Sandoval and Molitoris 2004).

The more common adverse effects associated with aminoglycosides include gastrointestinal adverse reactions which are usually mild and resolve upon drug discontinuation. Hypersensitivity to the aminoglycosides is extremely rare and is generally not observed even with direct instillation into the CNS. Neuromuscular blockade is a rarely reported adverse effect of aminoglycosides. It is more likely to occur when the aminoglycoside is given intravenously concurrently with a neuromuscular blocking agent or other anesthetic agent. Despite the concern for increased risk of neuromuscular blockade with the administration of the high doses routinely used in once-daily dosing protocols, this adverse event has not been observed (Gilbert 1991; Nicolau et al. 1995). Overall, the aminoglycosides are generally well tolerated; however, the major impedance to their use is the potential for ototoxicity and nephrotoxicity.

Ototoxicity is a well-known adverse effect of aminoglycosides and has a reported incidence between 2 and 25 %. It manifests as either auditory (cochleotoxicity) or vestibular toxicity and may occur alone or simultaneously (Govaerts et al. 1990). The mechanism of injury is similar with both manifestations and involves damage to the sensory hair cells in the cochlea and labyrinth (Hutchin and Cortopassi 1994). Unlike nephrotoxicity, ototoxicity is irreversible and is difficult to detect early, before the onset of symptoms occur.

Auditory toxicity is an adverse effect of aminoglycosides that is infrequently reported by patients, usually due to factors such as illness or surgery. When audiometry testing is performed in the high-frequency range, toxicity is more commonly reported. Patients often complain of tinnitus, which may be transient, and of a feeling of fullness in their ears (Fausti et al. 1992). The relationship between auditory toxicity and pharmacodynamics has suggested that toxicity is related to the AUC that occurs in the cochlear perilymph (Beaubien et al. 1991). This AUC is proportional to the area under the plasma curve and this data would suggest that regimens that use the same total daily dose have the same incidence of ototoxicity. Meta-analysis data support this by demonstrating that when hearing loss rates were determined audiometrically, there is no difference between once-daily dosing and multiple dose regimens (Contopoulos-Ioannidis et al. 2004). There is a lack of well-controlled data to detect differences in ototoxicity among aminoglycosides after systemic administration, there are some data that reports relative comparisons among aminoglycoside groups; gentamicin, kanamycin, and tobramycin tend to be more cochleotoxic than amikacin (Govaerts et al. 1990).

Similar to the auditory symptoms, the vestibular symptoms of aminoglycoside therapy are rarely reported due to the nonspecific nature of the initial presentation (i.e., nausea, vomiting, cold sweats, nystagmus, vertigo, and dizziness) (Federspil 1981). While considered to be less frequent than auditory toxicity, these vestibular effects are irreversible and therefore may have a profound impact on the daily function status of the patient. In contrast to the auditory toxicity potential of the aminoglycosides, streptomycin is thought to be more vestibulotoxic than gentamicin, which is more toxic than tobramycin. Once again, the vestibular toxicity of aminoglycosides is thought to be related to the total AUC of drug exposure, with no differences seen between once-daily dosing and multiple doses per day (Contopoulos-Ioannidis et al. 2004).

Due to the permanent nature of the ototoxicities associated with the aminoglycosides, efforts are made to reduce these toxicities through the application of pharmacodynamic principles. Although serum concentration data may be useful to ensure an adequate pharmacodynamic profile, these data cannot accurately predict the development of ototoxicity. Recent data suggested that toxicity is related to drug accumulation within the ear, not peak concentrations. These data support the concept of saturable transport and reinforce the belief that higher peak concentrations should not result in increased ototoxicity (Beaubien et al. 1991). For these reasons, once-daily administration techniques may minimize drug accumulation and therefore drug-related toxicity (Proctor et al. 1987; Rybak et al. 1999).

Aminoglycosides are considered obligate nephrotoxins and all patients will manifest this toxicity if maintained on an aminoglycoside regimen for a long enough time (Begg et al. 1992). The mechanism of renal toxicity is due to the accumulation of the aminoglycoside within the proximal tubular epithelial cells in lysosomal phospholipid complexes, which eventually rupture and cause cell death (Moore et al. 1984c). Because of this cell death the local renin–angiotensin system is activated and local vasoconstriction occurs causing a decrease in the glomerular filtration rate (Drusano and Louie 2011). Measures of this toxicity include the increase of serum creatinine values and decrease in glomerular filtration rate (Begg et al. 1992).

Uptake of the aminoglycosides into the tubular epithelium is a saturable process and therefore the increasing concentrations that occur with higher doses do not result in greater rates of uptake or greater toxicity. Investigators have theorized that once-daily dosing of aminoglycosides allows for a period in which the drug can leach back into the lumen and reduce the rate of accumulation (Nicolau et al. 1995). The exact interaction between the drug concentration and nephrotoxicity is not known, but nephrotoxicity is more common when the trough concentrations are elevated (Begg et al. 1992). Studies have shown daily AUC values and the actual dosing schedule to be significant predictors of time to onset of nephrotoxicity (Drusano and Louie 2011). Several investigators have also reported that advanced age, preexisting renal dysfunction, hypovolemia, shock, liver dysfunction, obesity, duration of therapy, use of concurrent nephrotoxic agents or agents that reduce renal blood flow, and elevated peak/trough aminoglycoside concentrations are all risk factors for development of nephrotoxicity (Moore et al. 1984c; Sawyers et al. 1986; Whelton 1985; Bertino et al. 1993). Surprisingly, it has been found that nephrotoxicity varies according to the time of day at which the drug is administered. Administration during active periods (1:30 pm) has been shown to be associated with less nephrotoxicity compared with administration during rest periods (1:30 am) (Beauchamp and Labrecque 2001).

Fortunately, nephrotoxicity is reversible in most cases, with renal function returning to normal within 3–6 weeks. However, to reduce the risk of nephrotoxicity, many methods that may be employed. The first method is to modify the cellular interactions, either through the complexing of the aminoglycoside extracellularly or by decreasing the binding of the aminoglycoside to the brush-border membrane through the use of compounds which raise the urinary pH (Mingeot-Leclercq et al. 1999; Mingeot-Leclercq and Tulkens 1999). Secondly, protective agents which include antioxidants (i.e., deferrioxamine, methimazole, Vitamin E, Vitamin C, or selenium) and certain antibiotics (i.e., ceftriaxone or daptomycin) may ameliorate nephrotoxicity (Rougier et al. 2004). Finally, dosing methods to enhance the principles of pharmacokinetics and pharmacodynamics may be adopted. Due to the presence of the saturable aminoglycoside transport system which is pivotal to the development of nephrotoxicity, less frequent single-daily dose administration may minimize accumulation and nephrotoxicity (Verpooten et al. 1989). In this regard, once-daily regimens have been reported to reduce the incidence of nephrotoxicity (Nicolau et al. 1995; Rybak et al. 1999; Murray et al. 1999).

In summary, the mechanisms of aminoglycoside ototoxicity and nephrotoxicity have been well studied and it has been seen that the application of pharmacodynamic principles, such as implementing once-daily aminoglycosides may ameliorate the toxicity of aminoglycosides. However, further studies and explanations need to be sought out that fully describe the complex intracellular mechanisms involved in order to truly protect our patients while using these agents to their full potential safely.

Clinical Usage and Application of Pharmacodynamics

The parenteral aminoglycosides, particularly gentamicin, tobramycin, and amikacin, have long been used empirically for the treatment of the febrile neutropenic patient or the treatment of patients with serious nosocomial infections. Due to the emergence of multidrug-resistant Gram-negative organisms, it is common practice that β-lactams are not given alone to treat systemic Gram-negative infections empirically due to the fact that many organisms, in particular, *P. aeruginosa* are known to develop resistance during therapy. Despite their obvious toxicodynamics, the aminoglycosides continue to play an important role in combination therapy for Gram-negative infections.

As discussed earlier the aminoglycosides are also commonly utilized in combination with a cell wall active agent for synergistic purposes for Gram-positive infections. In this situation gentamicin is frequently administered to provide synergy in the treatment of serious infections due to *Staphylococci* spp., *Enterococci* spp., and *Viridans streptococci*.

Aminoglycoside administration employs the use of two different intravenous dosing techniques. The older of the two approaches is often called the "traditional method" and uses multiple administrations of smaller doses every 8 h. Using this technique, the dosing regimen is administered through the use of an initial loading dose, calculated based on ideal body weight and whose purpose is to achieve a rapidly therapeutic serum concentration, followed by maintenance doses. The goal of this method is to obtain a peak concentration many multiple of the MIC value of the organism and to maintain trough levels at or above this same MIC value. This method usually involves 1.7–2 mg/kg every 8 h for gentamicin and tobramycin, while amikacin was frequently dosed using regimens of 5 mg/kg every 8 h or 7.5 mg/kg every 12 h (Fig. 9.1). Often to simplify this method, nomogram-based methods are used instead of individualized pharmacokinetically derived doses for initial doses. However, the preferred method of dosage adjustment is to individualize the regimen using the standard pharmacokinetic dosing principles when aminoglycoside peak and trough concentrations are available (Sawchuk and Zaske 1976; Sawchuk et al. 1977; Mandell et al. 2005).

The second and more popular method is referred to as the once-daily, single-daily, or the extended interval dosing method (Fig. 9.1) (Schumock et al. 1995; Chuck et al. 2000). This method employs the pharmacodynamic properties of the aminoglycosides to maximize the efficacy of the agents while minimizing the

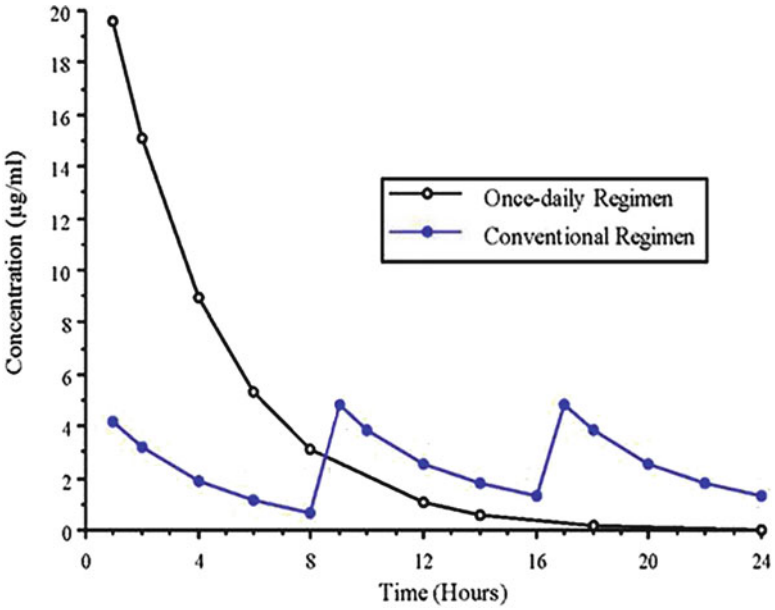

Fig. 9.1 Concentration-time profile comparison of conventional q8h intermittent dosing versus the once-daily daily administration technique

toxicity associated with their use. There are four distinct advantages of using extended dosing intervals for aminoglycosides (Gilbert 1991). As stated previously, giving aminoglycosides as a single-daily dose, as opposed to conventional strategies, provides the opportunity to maximize the peak concentration/MIC ratio and the resultant bactericidal activity (Fig. 9.1). Second, this administration technique minimizes drug accumulation within the inner ear and kidney and therefore decreases the potential for toxicities. Third, the PAE may also allow for longer periods of bacterial suppression during the dosing interval. Lastly, this aminoglycoside dosing approach may prevent the development of adaptive resistance.

Once-daily aminoglycoside therapy has been evaluated in multiple clinical trials including many patient populations (DeVries et al. 1990; Mauracher et al. 1989; Maller et al. 1991, 1993; Prins et al. 1993, 1994; Rozdzinski et al. 1993; TerBraak et al. 1990; International Antimicrobial Therapy Cooperative Group of the EORTC 1993; Beaucaire et al. 1991). Overall the conclusions of these trials have shown that the once-daily regimen is as efficacious as the traditional method of dosing but has the advantage of decreasing, but not eliminating, the risks of drug-induced ototoxicity and nephrotoxicity (Schumock et al. 1995; Demczar et al. 1997; Aggen et al. 2009). In addition, other advantages include being simpler, more cost effective, and less time consuming than the traditional method of dosing (Schumock et al. 1995). Lastly, several meta-analyses evaluating once-daily dosing with standard dosing regimens have shown that increased bacterial killing and trends for

decreased toxicity are actually demonstrated in clinical practice when the extended interval dosing is used (Galoe et al. 1995; Freeman and Strayer 1996; Hatala et al. 1996, 1997; Barza et al. 1996; Munckhof et al. 1996; Ferriols-Lisart and Alos-Alminana 1996; Bailey et al. 1997; Ali and Goetz 1997).

Studies have also been conducted in special populations including pediatrics (Marik et al. 1991; Nicolau et al. 1997; Sung et al. 2003; Mercado et al. 2004; Bhatt-Meht and Donn 2003; Dupuis et al. 2004; Piekarczyk et al. 2003; English et al. 2004; Botha et al. 2003; Kosalaraksa et al. 2004; Knight et al. 2003; Hansen et al. 2003) cystic fibrosis (Moss 2001; Ramsey et al. 1999) and pregnant populations (Bourget et al. 1991) for determination of serum concentrations, as well as comparisons for efficacy and safety between conventional administration and extended interval regimens. The majority of research has pointed to the international acceptance of extended interval dosing in infants and neonates including pre-term and full-term babies, although factors such as postnatal and gestational age and physiological status should be considered to determine patient-specific extended interval regimens. A meta-analysis by Contopoulos-Ioannidis et al. (2004) focusing on extended-interval administration of aminoglycosides in pediatric populations showed efficacy data measured as clinical failure rate, microbiologic failure rate, and combined effects favored once-daily dosing over traditional dosing with better safety profiles associated with the former.

Dosing regimens for once-daily dosing are unfortunately inconsistent in the published literature with large ranges in the various doses that have been used. Currently there are four commonly advocated methods for the administration of once-daily aminoglycosides. While each of these approaches differs somewhat with regard to dose and/or interval, all reflect the need for dosage modification in the patient with renal impairment. As of yet, no method has been shown to be superior over another, and while there may be concerns about the possible risk of increased toxicity with extended interval dosing in patients with reduced renal clearance, this risk is no greater than the risk associated with traditional dosing based on our current understanding of aminoglycoside-induced toxicity.

The first method of once-daily dosage determination is based on the pharmacokinetic and pharmacodynamic profile of these agents. This method, which was developed at Hartford Hospital, seeks to optimize the peak/MIC ratio in the majority of clinical situations by administering a dose of 7 mg/kg of either gentamicin or tobramycin (Nicolau et al. 1995). Similar to that of conventional regimens this protocol also provides modifications for patients with diminished renal function by administering a fixed dose with dosing interval adjustments for patients with impaired renal function (Nicolau et al. 1995). Due to the high peak concentrations obtained and the drug-free period at the end of the dosing interval, standard peak and trough concentrations are not drawn but rather a single random blood sample is obtained between 6 and 14 h after the start of the aminoglycoside infusion. This serum concentration is then used to determine the dosing interval based on the Hartford Hospital nomogram for once-daily dosing (Fig. 9.2) (Nicolau et al. 1995). While some studies have suggested that this nomogram may be inappropriate for the monitoring of therapy, a subsequent population pharmacokinetic analysis using data derived from more than 300 patients receiving 7 mg/kg of tobramycin further supports the

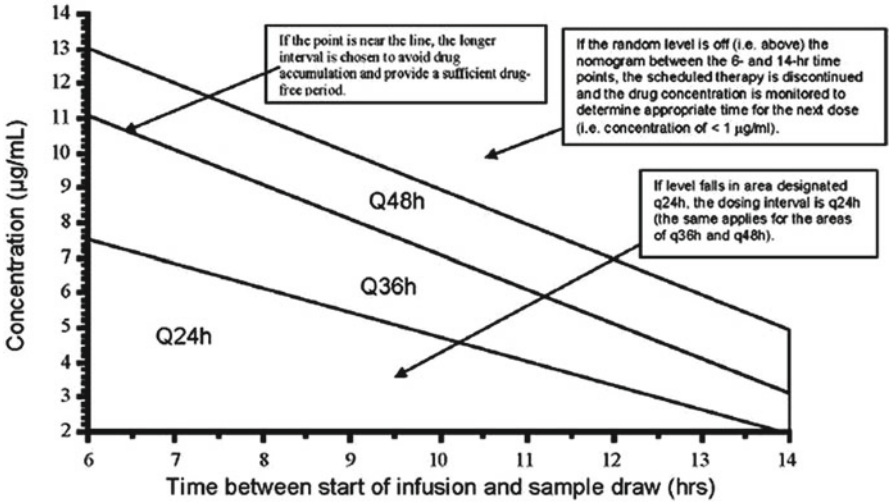

Fig. 9.2 Once-daily aminoglycoside nomogram for the assessment of dosing interval using a 7 mg/kg dose of gentamicin or tobramycin (Nicolau et al. 1995)

clinical utility of the original nomogram (Demczar et al. 1997; Xuan et al. 2000). Due to the low toxicity of this once-daily dosing regimen in addition to the usual short duration of therapy and the excellent renal function of most patients, some criteria have been developed to withhold the initial random concentration (which is obtained after the first or second dose) in patients who are (1) receiving 24 h dosing, (2) not receiving concurrent nephrotoxic agents, (3) without exposure to contrast media, (4) not quadriplegic nor amputee, (5) not in the intensive care unit, and (6) less than 60 years of age (Nicolau et al. 1995). In patients who meet the preceding criteria the serum creatinine should still be monitored at 2–3 day intervals throughout the course of therapy. For patients who continue on the once-daily regimen $\geq$5 days, a random concentration is obtained on the fifth day and weekly thereafter. Even though an initial random concentration may not be necessary in some patients, if a patient is experiencing rapidly changing creatinine clearances or has a creatinine clearance that is significantly reduced (i.e., $\leq$30 ml/min) it may be necessary to obtain several samples to adequately structure the administration schedule to maximize efficacy and minimize toxicity.

The second method for extended-interval dosing proposed by Gilbert utilizes a 5 mg/kg gentamicin or tobramycin dose in patients without renal dysfunction (Gilbert 1991; Gilbert and Bennett 1989). If the patient is experiencing diminished renal function, an adjustment in the dosing regimen may be made by modifying the dose and/or dosing interval in order to optimize therapy and minimize drug accumulation. A third method for extended interval dosing similar to that of Gilbert has been advocated by Prin and colleagues for patients with renal dysfunction (Prins et al. 1995). Finally, Begg and coworkers have suggested two methods to optimize once-daily dosing (Begg et al. 1995). The first is suggested for patients with normal renal

function and uses a graphical approach with target AUC values. The second method is targeted to patients with renal dysfunction and uses two aminoglycoside serum concentrations and a target AUC value based on the 24 h AUC value that would result with multiple-dose regimens for dosage modifications (Nicolau et al. 1996).

Extended-interval dosing was introduced into clinical practice with the purpose to optimize the pharmacodynamics of aminoglycosides in order to improve the clinical outcomes of patients receiving these agents for serious infections while reducing the incidence of drug-induced adverse events. In addition to meeting this goal, this dosing approach has also substantially reduced expenditures associated with the initiation of aminoglycoside therapy as compared to traditional dosing techniques (Nicolau et al. 1996; Hitt et al. 1997; Parker and Davey 1995).

Direct Delivery to the Site of Infection

For a drug to be effective, the agent must arrive and remain at the site of infection for enough time to disrupt the life cycle of the target pathogens. Application of pharmacodynamic principles suggests that in pneumonia, higher concentrations, especially peak concentration, at the target site of the lungs should correlate with improved efficacy (Klepser 2004; Flume and Klepser 2002). However, due to the properties of aminoglycosides, which includes their hydrophilicity, these agents may not achieve adequate or consistent local concentrations in certain sites such as bronchial fluids or bones and connective tissues. Therefore, a method of direct delivery to this site may enhance concentrations at the infection site and potentially reduce systemic toxicity. Utilization of aerolized tobramycin is the most prominent method to enhance efficient delivery to the intended site via direct local administration (Tiddens 2004; Cole 2001). Pharmacokinetic studies of inhaled tobramycin (TOBI®) have demonstrated significantly higher drug concentrations in the respiratory tract. In a randomized, double-blind, placebo-controlled study performed in cystic fibrosis patients, TOBI® significantly improved lung function, reduced sputum bacterial density, and decreased hospital stay (Moss 2001; Ramsey et al. 1999). Additionally, patients demonstrated fewer toxicities after administration of TOBI®. In addition to the proven efficacy for cystic fibrosis patients, treatment with inhaled tobramycin significantly reduces bacterial loads in patients with bronchiectasis and *P. aeruginosa* infections (Barker et al. 2000).

New Agents

Despite the decreasing numbers of novel agents with activity against Gram-negative organisms, recently work has been undertaken to discover and study new aminoglycosides. These newer agents have been studied in combination with cell wall active agents to combat drug-resistant Gram-positive bacteria such as methicillin-resistant

S. aureus, and as monotherapy against multidrug-resistant Gram-negative organisms. Although they have not yet been tested in clinical trials, the results seen in these preclinical trials have given hope to practitioners faced with the resistant organisms (Aggen et al. 2009; Zurenko et al. 2009; Lin et al. 2009).

Conclusion

The pharmacodynamic profile of aminoglycosides demonstrates that the activity of these agents is maximized when high dose, extended interval dosing regimens are employed. The use of this dosing technique has considerable in vitro and in vivo support, justifying the extensive use. The implementation of this regimen will maximize the probability of clinical cure, minimize toxicity, and may help to avoid the development of resistance. Although such dosing is not appropriate for all patients, this strategy appears to be useful in the majority of patients requiring aminoglycoside therapy and can be successfully employed as a hospital-wide program.

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Chapter 10
Continuous Infusion of Beta-lactam Antibiotics

Anouk E. Muller and Johan W. Mouton

Abstract For beta-lactam antibiotics continuous infusion can be used to optimise antibiotic therapy. Pre-clinical studies in rodents and in vitro studies have shown the benefits of continuous infusion when compared to intermittent dosing. Pharmacokinetic studies in humans have shown an improved probability of target attainment for continuous infusion. However, the relationship between continuous infusion and improved clinical outcome is ambiguous. The superiority of continuous infusion over intermittent dosing in clinical outcome studies is most often documented in special subgroups, such as critically ill patients or patients infected with less-susceptible micro-organisms. Methods to calculate doses during continuous infusion and practical issues, such as stability of antimicrobial solutions, are described.

Keywords Beta-lactams • Dose calculation • Prolonged or extended infusion • Continuous infusion • Pharmacodynamic target

List of Abbreviations

CF	Cystic fibrosis
CL	Clearance
C_{max}	Maximum concentration

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FDA	Food and Drug Administration
FEV1	Volume exhaled during the first second of a forced expiratory manoeuver started from the level of total lung capacity
FVC	Forced vital capacity; total amount of air that can forcibly be blown out after full inspiration
ICU	Intensive care unit
Iv	Intravenous
IVPM	In vitro pharmacokinetic–pharmacodynamic (PK/PD) models
MCS	Monte Carlo simulation
MIC	Minimum inhibitory concentration
MSW	Mutation selection window
PD	Pharmacodynamics
PD50	Daily dose required to protect 50 % of the animals from mortality
PK	Pharmacokinetics
PTA	Probability of target attainment
TBC	Total body clearance
Vd	Volume of distribution
$\%fT>MIC$	Percentage of time that the unbound concentrations is above the MIC

Introduction

The application of continuous infusion of beta-lactam antibiotics has been a matter of debate since the discovery of penicillin, when it was shown that more frequent dosing of penicillin resulted in a superior outcome compared to once or twice daily dosing (Eagle et al. 1950, 1953a, b; Schmidt and Walley 1951; Jawetz 1946; Schmidt et al. 1949). Nowadays, discussions on altering beta-lactam dosing to optimise antibacterial activity and maximise clinical outcomes are still ongoing and continuous infusion of beta-lactam antibiotics is not frequently used, although studies in in vitro system as well as in animals have clearly shown an advantage of continuous infusion over intermittent infusion. This may appear unfortunate as, despite the introduction of antibiotics into clinical practice over 60 years ago, the burden of infectious diseases remains high and with the increasing development of antibiotic resistance, optimisation of antimicrobial therapy is more than warranted. Improved antibiotic treatment has been shown to be of major importance, especially in critically ill patients with sepsis (Garnacho-Montero et al. 2003, 2006; Harbarth et al. 2003; Kollef et al. 1999).

There are several reasons that no definitive clinical benefit has been shown for the use of continuous infusion over intermittent infusion and, therefore, its limited use. The main reasons are the diversity of populations treated, the insufficient power of most studies and the use of concomitant additional active antimicrobials.

In this chapter, the pre-clinical and clinical evidence favouring continuous infusion of beta-lactam antibiotics is summarised. In addition, a number of practical issues are discussed that may help clinicians to provide this mode of administration.

PK/PD of Beta-lactams

Several pharmacokinetic/pharmacodynamic (PK/PD)-indices of antimicrobial agents have been described to correlate with efficacy or outcome (see Chap. 11). The most important PK/PD-index for beta-lactam antibiotics has been shown to be the duration of time over which the unbound drug concentration remains above the minimum inhibitory concentration (MIC; $\%fT > MIC$) (Vogelman et al. 1988; Drusano 1990; Craig and Ebert 1992; Muller 2013). This characteristic is used as an argument to administer beta-lactam antibiotics by continuous infusion. The evidence concerning this, both in in vitro studies as in animal studies, is discussed below.

In Vitro Pharmacodynamic Effects

The killing activity of beta-lactam antimicrobials against Gram-negative rods has been shown to be relatively slow and continues over time. In addition, maximum killing is attained at relatively low concentrations. Figure 10.1 shows an example of time–kill curves of ceftazidime to illustrate this mode of action. *P. aeruginosa* is exposed to varying concentrations of ceftazidime—expressed as multiples of the Minimum Inhibitory Concentration (MIC, mg/L). The figure shows that concentrations higher than 4 mg/L or four times the MIC—the MIC of this strain was 1 mg/L—do not result in increased killing activity. Bacterial killing at concentrations greater than 4–5 times the MIC does not increase with Enterobacteriaceae, *Staphylococcus aureus* or *Pseudomonas aeruginosa* (Hyatt et al. 1995; Bowker

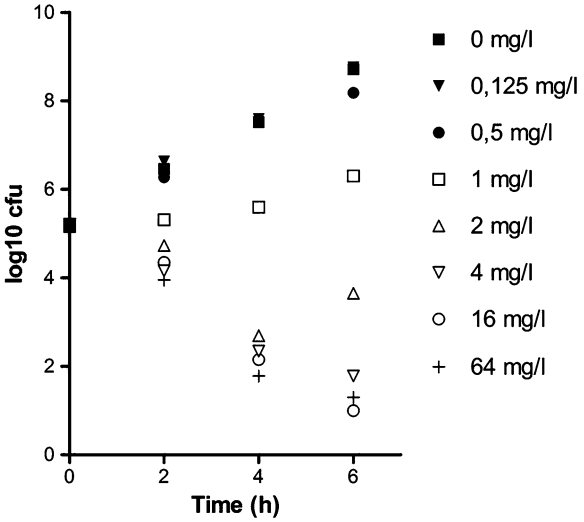

Fig. 10.1 Time–kill curve of ceftazidime. Modified from Mouton and Vinks (2007)

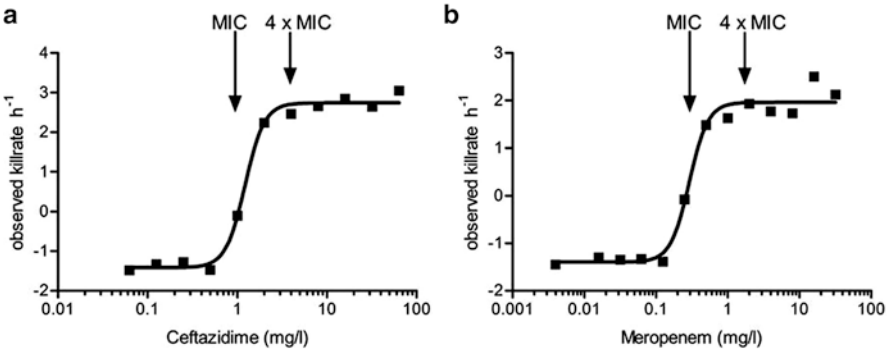

Fig. 10.2 Relationship between concentration of ceftazidime (**a**) and meropenem (**b**) and kill rate. The relationship follows a Hill type model with a relatively steep curve; the difference between no effect (growth, here displayed as a negative kill rate) and maximum effect is within 2–3 twofold dilutions. The maximum kill rate is attained at around 4×MIC. Figure modified from Mouton and Vinks (2005b, 2007). Reproduced from Mouton JW, Vinks AA. Pharmacokinetic/pharmacodynamic modelling of antibacterials in vitro and in vivo using bacterial growth and kill kinetics: the minimum inhibitory concentration versus stationary concentration. Clin Pharmacokinet. 2005;44(2):201–10 with permission from Adis (© Springer International Publishing AG [2005]. All rights reserved

et al. 1996; Mouton and Vinks 2005b). Slight differences in degree and rate of killing may exist between penicillins, cephalosporins and carbapenems, with carbapenems being most rapidly bactericidal and penicillins least against Gram-negative pathogens (Periti and Nicoletti 1999). In contrast to the beta-lactams, several other antibiotics, including aminoglycosides, show a clear concentration-dependent killing, in that killing of bacteria increases with increasing concentration (Vogelman and Craig 1986).

Another way to express the pharmacodynamic properties is to plot the rate of kill derived from the kill curve experiments just described as a function of concentration (Mouton and Vinks 2005a). This is shown in Fig. 10.2 (Mouton and Vinks 2005a, b). Here from, it can be concluded that the maximum kill rate of ceftazidime and meropenem are reached at around four times the MIC. Since the maximum killing effect of beta-lactams is reached at four times the MIC and higher concentrations not further contributing to the increase of the antimicrobial effect, the postulate was and is that high peak concentrations after intermittent infusion do not contribute to efficacy, whereas prolonged concentrations below the MIC result in reduced efficacy. In contrast, continuous administration resulting in concentrations above the MIC, but preferably above four times the MIC during the whole dosing interval, should result in prolonged activity. In a simulation study, we demonstrated that efficacy is maximised when free drug concentrations are maintained at concentrations that result in maximum bactericidal activity, thus four times the MIC (Mouton and Vinks 2005b).

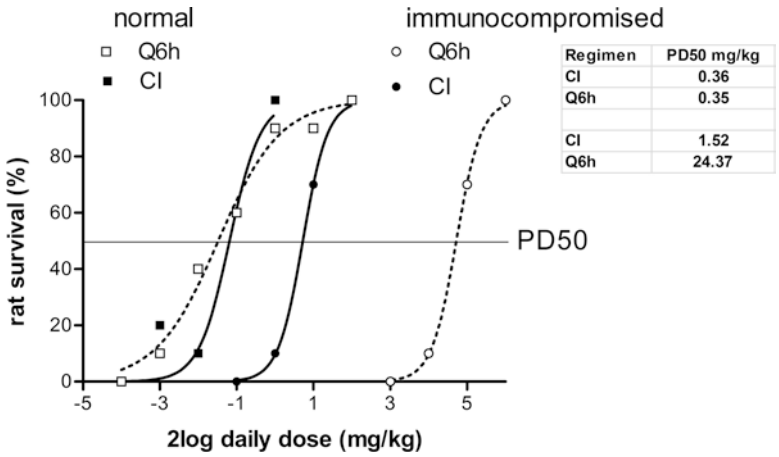

Fig. 10.3 Relationship between daily dose and mortality in a pulmonary infection models in rats. The PD50's for two different dosing regimens in immunocompetent as well as immunodeficient animals are also displayed. Efficacy of continuous infusion is higher than intermittent infusion in immunodeficient animals (Mouton and Vinks 2007)

Studies in Animals

After the early studies after the discovery of penicillin (Eagle et al. 1950, 1953a, b; Schmidt and Walley 1951; Jawetz 1946; Schmidt et al. 1949), it took several decades before investigators looked at the impact of the dosing frequency on outcome (Leggett et al. 1989, 1990). In an authoritative review by Craig and Ebert (1992), the authors compiled data from a number of animal studies and concluded that more frequent administration leads to significant improved efficacy, thereby substantiating the conclusions derived from in vitro studies. Studies on continuous infusion itself were, because of experimental difficulty, relatively few. Extensive studies comparing continuous infusion with intermittent infusion were performed by the group of Bakker-Woudenberg (Roosendaal et al. 1985, 1986, 1987, 1989; Roosendaal and Bakker-Woudenberg 1990). Using a neutropenic rat model of infection and a treatment duration of 4 days, these investigators showed that continuous infusion of ceftazidime was superior to intermittent dosing (Fig. 10.3). The daily dose required to protect 50 % of the animals from mortality (PD50) was 1.5 mg/kg with continuous infusion and 24.4 mg/kg during the q6h dosing regimen, a factor 15 difference. However, when the effects of the dosing regimens in non-neutropenic animals were compared, PD50s (0.35 and 0.36 mg/kg) for both dosing regimens was lower than in immunodeficient mice, and the difference between the two modes of administration almost completely disappeared. These data indicate that continuous infusion could be particularly useful in patients with an impaired immune system. Alternatively, the difference in effect between continuous and

intermittent infusion in immunocompetent animals is virtually non-existent and this may help explain, in part, why clinical trials have so far failed to show significant differences in clinical outcome. In contrast, Croisier and colleagues (2008) compared continuous versus intermittent infusion in a pharmacokinetically humanised immunocompetent rabbit model and found that continuous infusion was more efficacious than intermittent infusion. In addition, they found an additive or synergistic effect of tobramycin during continuous infusion but not during intermittent infusion. Continuous versus intermittent infusion of cefazolin in different surgical infection models was studied by Naziri et al. (1995) and Livingston and Wang (1993). In both studies, continuous infusion performed significantly better than intermittent infusion. A different approach in comparing the two modes of administration was taken by Buijs et al. (2007). They looked at endotoxin release and other inflammatory markers during and after administration of ceftazidime and meropenem and concluded that the mode of administration as well as the beta-lactam significantly affected these markers.

In conclusion, pre-clinical studies in rodents have clearly shown the benefit of continuous infusion.

Studies in In Vitro Models Simulating Human Pharmacokinetics

Although the results of animal studies clearly indicate that continuous infusion is superior in neutropenic animals, one of the counterarguments is that the half-life of beta-lactams (and other drugs) in rodents is much shorter than in humans and therefore the impact of more frequent dosing more pronounced in these animals. In vitro pharmacokinetic–pharmacodynamic (PK/PD) models (IVPM) have been used to expose bacteria to human concentration time profiles to overcome this problem. In one of our earlier studies, we showed that continuous infusion of ceftazidime was more efficacious than ceftazidime given three times daily against *P. aeruginosa* (Mouton and den Hollander 1994). However, resistant strains emerged when concentrations were near the MIC, and killing was also less pronounced than at concentrations of four times the MIC. Other authors have confirmed these results (Alou et al. 2005; Cappelletty et al. 1995). Since the effect of the immune system is absent in the IVPM, the results are likely to be more in line with those found in immunodeficient individuals than immunocompetent individuals.

Conclusions from Pre-clinical Models

From these pre-clinical studies, it can be concluded that high peak concentrations do not contribute to the efficacy of beta-lactams, and killing proceeds over time. For an optimal killing effect, an antibiotic concentration of four times the MIC is desired during the entire dosing interval.

Comparative Studies in Humans

Comparative studies in humans can be divided in three categories. In the first, the PK of beta-lactams is compared during dosing regimens with continuous and intermittent infusions. The primary aim of these studies was to compare the time–concentration profiles of the different modes of administration and calculate differences in $\%fT>MIC$. On the other hand, there are studies comparing clinical outcome between patients receiving intermittent or continuous infusion. Finally, many feasibility studies have been performed, and the comparison in regimens is primarily based hereon.

Pharmacokinetic Studies

A significant number of studies have appeared that have looked at the pharmacokinetics of different beta-lactams during continuous and intermittent infusion in volunteers (Mouton et al. 1990; Mouton and Michel 1991; Burgess and Waldrep 2002; Burgess et al. 1999, 2000), and various patient populations (Leder et al. 1999; Berkhout et al. 2003), including neutropenic patients (Pea et al. 2005; Dalle et al. 2002; Daenen et al. 1995), patients with cystic fibrosis (Kuti et al. 2004; Riethmueller et al. 2009; Hubert et al. 2009; Vinks et al. 1994, 1996a, 1997, 2003) and patients in the Intensive Care Unit (Buijk et al. 2002, 2004b; Benko et al. 1996; Hanes et al. 2000; Roos et al. 2007; Rafati et al. 2006; Lipman et al. 1999). For specific groups of patients, such as ICU patients, pharmacokinetic profiles might differ from other patients. Although studies performed in ICU patients clearly show pharmacokinetic properties that differ from those of non-ICU patients, mainly due to an increased volume of distribution and a lower clearance, significant differences within the same groups of patients have seldom been found between the two modes of administration. In general, $\%fT>MIC$ in patients treated with continuous infusion was found to be longer compared to $\%fT>MIC$ in patients treated with intermittent doses. Therefore, theoretically the efficacy of beta-lactams is increased with continuous infusions and this is the conclusion in most of the papers.

In addition to the variability seen between patients and different patient groups, intraindividual variability in patients at different times of the days was also observed. In the study by Buijk et al. (2002), we determined the pharmacokinetic profile of ceftazidime in the ICU setting. Figure 10.4 shows the concentrations of ceftazidime at two time points, in the morning (8 a.m.) and at the end of the afternoon (4 p.m.). Not only is there significant variation between patients but also significant variation during the day. This relatively large individual variability has also been observed by others (Pea et al. 2005; Hanes et al. 2000; Georges et al. 2005; De Jongh et al. 2008). In particular, in the study by De Jongh, a large variability was observed during the day (De Jongh et al. 2008). In addition, these authors found a significant variability in protein binding further contributing to the variability of active drug

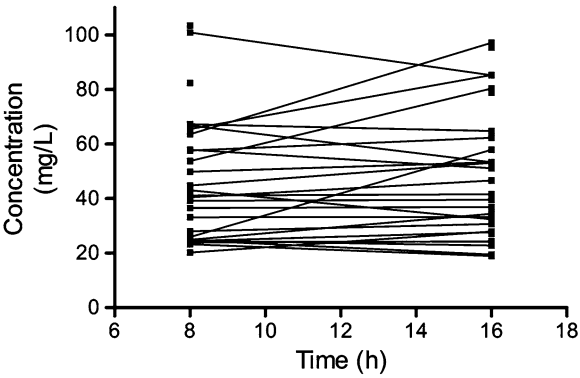

Fig. 10.4 Concentrations of ceftazidime during continuous infusions of ceftazidime (4.5 g/day) in patients on the ICU. Unpublished data from Mouton and Vinks (2007)

concentrations. In children, concentrations during continuous infusion of cefotaxime varied widely between patients age 0–17 years, especially in children younger than 1 week (Bertels et al. 2008).

The variability over the day does not explain the differences in profiles between the dosing groups, but the large variability observed raises the question perhaps whether therapeutic drug monitoring is warranted in these patients. So far, this has not been routinely implemented for beta-lactam agents.

Efficacy Studies

Statistically significant differences in clinical outcome favouring continuous infusion are most likely to occur if the concentration at steady state is higher than the trough concentration during intermittent infusion and the trough concentration is below the MIC. At low MICs, even intermittent dosing regimens will result in 100 % $fT > MIC$ and differences are not likely to be present. Since MICs of microorganisms differ in patients, the benefit of continuous infusion will not always be obvious and the power of these comparative studies is thus relatively low. Even then, the number of randomised controlled trials or randomised cross-over studies aimed to evaluate continuous infusion versus intermittent infusion is limited (Table 10.1). In addition, a second antibiotic was used in 7 of the 12 studies mentioned in the table, and five of those consisted of high-dose aminoglycosides (see table for details). The number of patients in most studies is relatively small, with studies generally designed to either show non-inferiority or set-up as a pilot study. No comparative studies are available in the general paediatric population and studies in specific populations are discussed in that paragraph.

The first larger study that did look at efficacy in patients was by Bodey et al. (1979). These investigators compared continuous infusion of cefamandole versus intermittent infusion. A third arm of the study involved continuous infusion of tobramycin and all groups also received carbenicillin. While no difference in

Table 10.1 Randomised (controlled) studies comparing outcome during continuous infusion and intermittent administration

Study	Drug[a]	Regimen[b]	*N* CI/*N* Int[c]	Indication	Outcome measure	Outcome	Remarks
Bodey et al. (1979)	Cefamandole (+carbenicillin 5 g q6h)	12 g CI vs. 3 g q6h	74/92[d]	FUO in neutropenic patients	Clinical cure	NS	$p=0.03$ in favour of CI for infections in patients with persistent neutropenia
Nicolau et al. (2001)	Ceftazidime (+tobramycin 7 mg/kg qd)	3 g CI vs. 2 g q8h	17/18	Nosocomial pneumonia	Clinical cure/improvement; microbiological response	NS	Lower dose during CI
Georges et al. (2005)	Cefepime (+amikacin 15 mg/kg qd	4 g CI vs. 2 g q 12 h	26/24	Critically ill	Mortality; clinical cure; duration of mechanical ventilation; length of stay ICU	NS	
Hanes et al. (2000)	Ceftazidime	60 mg/kg CI vs. 2 g q8h	17/15	Nosocomial pneumonia	Clinical cure	NS	Lower dose during CI
van Zanten et al. (2007)	Cefotaxime	2 g CI vs. 1 g q8h	47/46	COPD exacerbation	Clinical cure	NS	Lower dose during CI
Lau et al. (2006)	Piperacillin/ tazobactam	12/1.5 g CI vs. 3/0.375 g q6h	130/132	Complicated intra-abdominal	Mortality; clinical cure; adverse effects	NS	
Roberts et al. (2007)	Ceftriaxone (+undefined other antibiotics)	2 g CI vs. 2 g q24 h	29/28	Intensive care	Mortality; duration of mechanical ventilation; length of stay ICU or hospital	NS	$p=0.008$ in favour of CI in a priori analysis after logistic regression $p=0.02$ in favour of CI for proven bacterial eradication
Buijk et al. (2002)	Ceftazidime	4.5 g CI vs. 1.5 g q8h	12/6	Intensive care	Mortality	NS	

(continued)

Table 10.1 (continued)

Study	Drug[a]	Regimen[b]	*N* CI/*N* Int[c]	Indication	Outcome measure	Outcome	Remarks
Rafati et al. (2006)	Piperacillin (+amikacin 15 mg/kg qd)	8 g CI vs. 3 g q6h	20/20	Septic critically ill	Mortality; decrease in disease severity; duration of pyrexia, normalisation white blood count	NS	Temporarily better APACHE II scores for CI at day 2, 3 and 4 ($p \leq 0.04$)
Riethmueller et al. (2009)	Ceftazidime (+tobramycin 10 mg/kg qd)	100 mg/kg vs. 200 mg/kg divided over 3 doses	56/56[e]	CF patients colonised with *P. aeruginosa* (elective treatment)	White blood count normalisation; adverse effects	NS	Randomised cross-over study
Hubert et al. (2009)	Ceftazidime (+tobramycin 10 mg/kg qd)	200 mg/kg (max 12 g) vs. 200 mg/kg (max 12 g) divided over 3 doses	54/54[e]	Pulmonary exacerbation in CF patients colonised with *P. aeruginosa*	Change in FEV1; adverse effects	NS	Randomised cross-over study; subgroup of patients colonised with resistant *P. aeruginosa* strains: in favour of CI ($p < 0.05$)
Dulhunty et al. (2013)	Piperacillin/ tazobactam Meropenem Ticarcillin/ clavulanate	13.5 g CI[f] 3 g CI[f] 12.4–13.5 CI[f]	30/30	Intensive care	Clinical response after 7–14 days ICU-free days at day 28 Hospital survival	$p=0.037$ $p=0.14$ $p=0.47$	$p=0.037$ in favour of CI

NS not significant, *CI* continuous infusion

[a] Loading doses not tabulated

[b] Dose refers to dose per 24 h

[c] Numbers refer to intend to treat

[d] Number of febrile episodes

[e] Number of episodes used in analyses

[f] Total daily dose for intermittent dosing identical to amount of drug administered for CI. No further information

outcome was found between the groups receiving cefamandole as a continuous or intermittent infusion, a subanalysis focussing on patients with persistent neutropenia did show an advantage of continuous infusion. A second study that did show a difference in clinical outcome was a study by Roberts et al. (2007) that compared continuous infusion of ceftriaxone with a once daily regimen as a pilot study. While no differences in overall outcomes were found in the intention to treat analysis, an analysis of patients defined a priori as evaluable or patients with a low APACHE II score, showed a distinct advantage towards continuous infusion in the multiple logistic regression analysis ($p=0.008$). The authors concluded that a large-scale study is warranted. The reason why these authors did find a difference while the majority of the other studies did not could be that once daily dosing of ceftriaxone in the ICU setting is indeed inadequate in that the $\%fT>MIC$ is too short. The authors did not determine this, however. Another study showed a difference in clinical outcome between intermittent dosing and continuous infusion for piperacillin. However, the differences were not throughout the entire study period. The advantage of continuous infusion on the APACHE II score was temporarily on the second to fourth day (Rafati et al. 2006). On the fifth and sixth day, no differences were demonstrated. Finally, the study of Hubert et al. compared ceftazidime intermittent dosing versus continuous infusion in CF patients colonised with *P. aeruginosa* (Hubert et al. 2009). Although no differences between the two treatment groups were seen initially, a subanalysis indicated that the clinical outcome after continuous infusion was superior compared to after intermittent dosing for patients colonised with *P. aeruginosa* strains that were resistant to ceftazidime. Recently, Dulhunty et al. (2013) performed a double-blind, randomised controlled trial of continuous infusion versus intermittent bolus dosing of piperacillin/tazobactam, meropenem, and ticarcillin/clavulanate in ICU patients. They found a higher clinical cure in the continuous infusion group (70 % vs 43 %, P=0.037), but no difference in ICU-free days nor in hospital survival. Some of the other studies showed advantages in favour of continuous infusion, though not statistically significant or significant only in subanalysis, but none of the studies showed beneficial effects for intermittent dosing.

Of the non-randomised studies, the study of Lorente et al. (2006) showed a significant difference in cure rate between meropenem treatment groups in favour of continuous infusion (OR 6.44 (95 % CI 1.97–21.05); $p<0.001$) in a retrospective cohort study comprising patients treated for ventilator-associated pneumonia due to Gram-negative bacilli. All patients ($N=89$) received tobramycin concomitantly. They performed a similar study for piperacillin/tazobactam ($N=83$). This study was also performed in patients treated for VAP and they found a significant difference in clinical outcome between patients in which the drug was administered continuously versus intermittent dosing. However, the advantages were only present in patients treated for infections with micro-organisms with an MIC ≥ 8 mg/L (Lorente et al. 2009) (MIC 8 mg/L OR = 10.79; 95 % CI 1.01–588.24; $p=0.049$ and MIC 16 mg/L OR = 22.89; 95 % CI 1.19–1880.78; $p=0.03$). Grant et al. compared efficacy of piperacillin/tazobactam in a large community hospital time during continuous and intermittent infusion (Grant et al. 2002) ($N=98$). Although there was no difference in clinical success rates between the two groups ($p=0.081$), days to normalisation of temperature were significantly lower in the continuous infusion group ($p=0.012$). In addition, the costs of the continuous infusion regimen were lower.

Feasibility Studies

A large number of studies were designed to look at the feasibility of continuous infusion. This includes both the mode of administration itself as well as the pharmacological properties of the drug. Administration of a continuous infusion requires a suitable pump designed to deliver the drug at constant flow rate. In the hospital setting, several pump types are available. However, continuous infusion is being applied using portable pumps as well, enabling patients to remain ambulatory (Vinks et al. 1994, 1996b, 1997; Zeller et al. 2009; Walton et al. 2007). In particular, continuous infusion is being used during home treatment, because continuous infusion precludes the necessity of changing infusion bags repeatedly during the day, as used to be necessary during intermittent infusion, provided the drug is stable (see below). In the hospital setting, the use of portable pumps delivering a continuous flow will reduce the workload of nurses for the same reasons.

An important potential pitfall, which is often neglected, was described by Claus et al. (2010). Electronic pumps used in the extended and continuous infusion of beta-lactams have an infusion dead line space. The infusion line dead space might result in incomplete administration of the drug. Especially, patients with a fluid restriction for whom the antibiotics are administered in small amount of fluids are at risk. Claus et al. (2010) described that every replacement of the infusion line resulted in a 40 % loss of the prescribed antibiotic dose if the infusion line was not cleared with a compatible solution after the antibiotic infusion. On the other hand, non-replacement of the infusion line dead space increased the risk of infusion of the degraded product, in particular in view of the issue of stability of meropenem in solution, as the residual volume was infused in the first 75 min of the subsequent 3-h infusion. Replacement of dead space volume is therefore a critical issue. Attention has to be paid to this issue and the use of the more expensive pressurised pumps, where the infusion dead space is less than 1 mL, might be necessary for ICU patients, in particular for (premature) neonates, and patients with fluid restrictions (Claus et al. 2010).

Stability

The stability of an antibiotic in solution is an important consideration when choosing beta-lactam antibiotics for continuous infusion (Stiles et al. 1995; Gilbert et al. 1997; Leggett 2000). Drugs used for continuous infusion are often administered using a portable pump resulting in exposition of the solution to various temperatures, including room temperature. Apart from the reduction in active drug, this may lead to the formation of degradation products and may increase the frequency of hypersensitivity reactions, as has been described for penicillin-G (Neftel et al. 1982, 1984).

Drugs that are stable at room temperature for less than 8 h are best administered by intermittent dosing or prolonged infusion if possible. It is important to prepare the solution just before the start of the infusion and to refresh the antibiotic solutions regularly. Table 10.2 provides the stability for drug solutions. Amoxicillin and imipenem are stable at room temperature for 4–8 h only and refrigerated storage is not recommended. Meropenem and cefuroxime have better stability yet have to be replaced every 8–12 h. Cold ice packs have been used to increase the stability of meropenem in order to allow replacement every 24 h (Kuti et al. 2004). In contrast, anti-pseudomonal beta-lactams for continuous infusion, such as ceftazidime, cefpirome, cefepime and aztreonam, are stable at room temperature for at least 24 h and when refrigerated for at least 4–7 days (Vinks et al. 1996b; Stiles et al. 1989; Viaene et al. 2002; Baririan et al. 2003; Servais and Tulkens 2001; Sprauten et al. 2003; Florey 1988) and cefotaxime is also stable for 24 h. Prolonged exposure to temperatures above 25 °C however may drastically reduce stability making several these antibiotics less suited for delivery via an ambulatory pump carried close to the body or underneath clothing (Viaene et al. 2002; Baririan et al. 2003; Servais and Tulkens 2001). For several other β-lactams used in the ICU setting, stability of intravenous fluids for prolonged infusion is not well documented (Trissel 2006). Before this information becomes available, these solutions should be considered not suited for continuous infusion.

Toxicity

Differences in toxicity and adverse effects between intermittent dosing and continuous infusion have not been described. Most adverse effects reported during either treatment were minor and most of them related to the gastrointestinal tract. Studies report highly variable percentages of adverse effects, ranging from 0 % (Riethmueller et al. 2009; Benko et al. 1996; Thalhammer et al. 1999) to 72 % for ceftazidime in CF patients (Hubert et al. 2009). Most frequently reported adverse effects are abdominal pain, nausea, diarrhoea, hemoptysis, rash and headaches. Serious side effects, such as pulmonary exacerbations and seizures, are uncommon and there is also no difference in occurrence between continuous and intermittent infusion.

Arguments Against Continuous Infusion

One of the arguments against the use of continuous infusions for beta-lactam antibiotics is that there is no FDA approval for this dosing strategy. In some countries, continuous infusion ceftazidime is registered for the treatment of infectious exacerbations in patients with CF, for instance The Netherlands. However, the bacteria have evolved since the early clinical trials used to obtain FDA approval, and those outdated studies do not address the resistance profiles currently observed in clinical

Table 10.2 In vitro stability of β-lactam antibiotics in solutions for intravenous infusion during storage at different temperatures

Antibiotic	Concentration (mg/mL)	Diluent	Temperature at 37 °C (h)	Room temperature at 25 °C (h)	Refrigerated at +5 °C (days)	Frozen at −20 °C (weeks)	Reference
Amoxicillin	10–20	NS	–	8	1	2 (below −30)	Trissel (2006)
	30	NS	–	3	Not recommended		
Amoxicillin with clavulanic acid	10	SW, NS	–	4	8	Not recommended	Ashwin et al. (1987)
Aztreonam	100	NS, SW	24–36	–	8	24	Vinks et al. (1996b)
	100	SW	>24[a]	–	–	–	Viaene et al. (2002)
Benzylpenicillin	16 MU	NS	5	12–13	7	–	Vella-Brincat et al. (2004)
Cefamandole	100	DW5, NS, SW	–	24 (protect from light)	4	24	Trissel (2006)
Cefepime	24	DW5	–	24	5	14	Sprauten et al. (2003)
	50	SW	13	20	–	–	Viaene et al. (2002)
	50–120	SW	<12	24	–	–	Baririan et al. (2003)
Cefpirome	32	SW	7	23	–	–	Viaene et al. (2002)
Ceftazidime	100–180	DW5	–	24	4–7	12	Servais and Tulkens (2001), Trissel (2006), Stiles et al. (1992) Viaene et al. 2002)
	100–180	NS, SW	8	24	7	12	
Cefuroxime	22.5–45	SW	–	8–12[b]	7	4	Trissel (2006)
Doripenem	5	NS	12/8[c]	16[6]	–	–	Keel et al. (2011)
	10	PF	~12	24[a]	–	–	Berthoin et al. (2010)
	5	NS	–	12	3	–	Psathas et al. (2008
	5	D5W	–	4	2	–	Psathas et al. (2008)
Flucloxacillin	100–125	SW	–	24	3	12	Trissel (2006)

Imipenem with cilastatin	2.5–5	DW5	–	4	1	Not recommended	Trissel (2006)
	2.5–5	NS	–	10	2	Not recommended	Keel et al. (2011)
	5	NS	4/3[c]	6[b]	–	–	Viaene et al. (2002)
	8	SW	~3	3.5	–		
Meropenem	100	SW	–	8–12	2	4	Kuti et al. (2004), Mathot et al. (1997)
	30	NS	–	12–16	–	–	
	5	NS	8/6[c]	12[b]	–	–	Kuti et al. (2004)
	40	PF	6	–	–	–	Keel et al. (2011)
	60	PF	–	6	–	–	Berthoin et al. (2010)
	64	SW	1.5	5	–	–	Berthoin et al. (2010)
							Viaene et al. (2002)
Piperacillin	20–80; 200–500	D5W, NS, SW	–	24–48	7	4	Trissel (2006), Service (2005)
			22	30	–	–	
	128	SW					Viaene et al. (2002)
Piperacillin with tazobactam	80–200	D5W, NS, SW	–	24	7	4	Trissel (2006)
	128		–	12[d]	–	–	Viaene et al. (2002)
		SW	>24	>72	–	–	
Temocillin	83.4	SW	–	24[a]	–	–	De Jongh et al. (2008)
Ticarcillin	200–300	D5W, NS, SW	–	48	3	12	Trissel (2006), Service (2005)
Ticarcillin with clavulanic acid	10–100	NS, D5W, SW	–	48	3	4	Trissel (2006), Service (2005)

Antibiotic stability and compatibility under the specified conditions indicate the period for which a minimum of 90 % of the drug remains intact and available for delivery (*i.e.* decomposition of 10 % or less)

NS normal saline (NaCl 0.9 %), *D5W* dextrose 5 %, *SW* sterile water for injection, *PF* pyrogen-free water for injection

[a]Tested for 24 h

[b]At 30 °C

[c]At 35/40 °C

[d]In ambulatory infusion pump

practice (Lodise and Butterfield 2011), nor was it realised that pharmacodynamic properties of antimicrobial agents are important in defining a dosing regimen.

Some beta-lactams have a relatively long half-life, for instance ertapenem and ceftriaxone. The long half-life would ensure a $\%fT > MIC$ that is close to 100 % even without continuous infusion. The major reason for the long half-life of these agents is the high degree of protein binding. For example, ertapenem is highly albumin bound (85–95 %) in contrast to meropenem (2 % albumin bound), which considerably extends its elimination half-life. Ertapenem is therefore administered only once daily. However, several studies have shown that hypoalbuminaemia has considerable impact on the pharmacokinetics of ertapenem. Burkhardt et al. (2007) showed that the Vd in critically ill patients in the early phases of VAP had a Vd, which was doubled as compared to volunteers. The CL was also increased. Brink et al. (2009) confirmed these findings in critically ill patients with sepsis and Boselli also confirmed the findings in patients with a ventilator-associated pneumonia (Boselli et al. 2006). For ceftriaxone, similar arguments exist. Ceftriaxone has been used as continuous infusion in a comparative trial and was shown to have a benefit over intermittent infusion (Roberts et al. 2007).

An important argument against continuous infusion is the emergence of resistance during treatment, which is an increasing problem (Peterson 2005). Resistance can emerge in both the infecting target organism(s) and/or in the colonising normal flora. During treatment of infection the host's normal flora is unintentionally exposed to antibiotics, which may lead to secondary colonisation by potentially pathogenic, often multiple antibiotic-resistant organisms (Safdar and Maki 2002; Donskey 2004). Prevention of the emergence of resistance during antibiotic therapy is important and therefore is an issue in selecting the optimal dosing regimen. Goessens et al. (2007) demonstrated that treatment with third-generation cephalosporins in the therapeutic range had a profound effect on intestinal colonisation with *Enterobacter cloacae* and emergence of resistance in an animal model of severe infection. A strong reduction of the ceftazidime-susceptible bacteria and, in some animals, selection of pre-existing ceftazidime-resistant mutants was described. These mutants originated from the ceftazidime-susceptible *E. cloacae* population initially present in the intestine. They also showed that frequent administration of relatively small doses resulted in more emergence of resistance than infrequent administration. The PK/PD index that predicted resistance selection was the period of time that ceftazidime plasma levels fell within the mutation selection window (MSW). Based on these data, a continuous infusion resulting in concentrations within the MSW would increase the likelihood of emergence of resistance with continuous infusion in many cases. In a study in an IVPM, continuous infusion yielding concentrations below 4×MIC resulted in emergence of resistance (Mouton and den Hollander 1994). In clinical studies, there is no evidence (yet) available that more resistant strains emerge during continuous infusion. Several studies evaluated the susceptibility of the bacterial strains using the two dosing regimen (continuous and intermittent dosing) and did not find changes in the susceptibility pattern (Hubert et al. 2009; Nicolau et al. 2001).

A major problem that may occur when applying continuous infusion is the number of intravenous access sites, because continuous infusion requires one of those and precludes the administration of other drugs. This can be a problem if drug solutions are incompatible with each other. One of the solutions to that problem is using extended infusion, discussed in the next paragraph.

An Alternative to Continuous Infusion: Extended Infusion

Since continuous infusion is complicated for some beta-lactam antibiotics due to limited stability or in some situations due to practical problems, another option is to use extended infusion. In contrast to conventional intermittent dosing with infusion times lasting 0.5–1 h, or even shorter infusion times, the administration of drugs using prolonged or extended infusions lasts 3–4 h, usually 50 % of the dosing interval (MacGowan 2011).

For several beta-lactam antibiotics, studies on the pharmacokinetics using extended infusion were performed. Tam et al. (2003) studied the PK of cefepime using different treatment regimens and performed MCS. They concluded that the standard dosing regimen using intermittent infusion resulted in low probability of target attainment (PTA). The PTA of cefepime could be improved extended or continuous infusions (Tam et al. 2003). For piperacillin–tazobactam, Felton et al. (2012) studied the non-linear PK in 11 hospitalised patients with nosocomial infections. Using MCS, they found that intermittent administration of 4 g piperacillin resulted in satisfactory target attainments for the most sensitive of organisms only, while extended infusions every 6 or 8 h reached a satisfactory target attainment of 94 and 82 % respectively, for an MIC of 16 mg/L. The finding that the extended-infusion piperacillin–tazobactam regimen was pharmacodynamically superior to the intermittent-infusion regimen was confirmed by Patel et al. in patients with a nosocomial pneumonia (Patel et al. 2010). They compared 4.5 g piptazobactam i.v. as a 30-min infusion every 6 h with 3.375 g piptazobactam i.v. as a 4-h infusion every 8 h. However, both regimens were associated with suboptimal probability of target attainment for MICs of ≥32 mg/L, irrespective of the renal function (Patel et al. 2010). In summary, the results of the Monte Carlo simulations suggest that changing medical practice from bolus dosing to an extended infusion would improve target attainment rates.

For prolonged infusion, no randomised trials are available, but a few non-randomised trials are discussed. Lodise et al. (2007) performed a cohort study in critically ill patients infected with *P. aeruginosa* to compare intermittent infusion of piperacillin–tazobactam (3.375 g infused over 30 min ever 4 or 6 h) with extended infusion (3.375 g infused over 4 h every 8 h). All *P. aeruginosa* strains were susceptible to piperacillin–tazobactam. In patients with APACHE II ($N=79$; 38 intermittent and 41 extended infusion), there was a significant difference in 14 days mortality (12.2 % vs. 31.6 %, respectively; $p=0.04$) and the length of hospital stay (21 days

vs. 38 days; $p=0.02$). However, the same group performed another study in patients with low disease severity of disease with infections with various Gram-negative bacteria. In this study, they did not find significant differences in the two treatment groups ($N=59$ for intermittent dosing and $N=70$ for continuous dosing) for the length of hospital stay and the 30-day mortality. Possible explanations are the fact that in the intermittent dosing group, the dose of piperacillin–tazobactam was often 4.5 g and the dosing was not adjusted for impaired renal function. Furthermore, a wide range of diseases and pathogens were included (Patel et al. 2009). The study of Yost and Cappelletty (2011) included 186 patients with extended infusion of piperacillin–tazobactam (infused over 4 h) in a retrospective study, but compared these data to data from 173 patients receiving conventional dosing of other beta-lactams with similar antibacterial spectrum. They found differences in mortality, but these data are difficult to interpret because the treatment groups were not fully comparable in the percentages of concomitant aminoglycoside use, positive cultures with *P. aeruginosa* and numbers of positive respiratory tract cultures. Wang compared two groups of patients treated for a hospital-acquired pneumonia with positive cultures with multidrug-resistant *Acinetobacter baumannii* (Wang 2009). He showed that meropenem 1 g q8h infused in 1 h resulted in similar clinical effects as meropenem 500 mg q6h infused over 3 h (Wang 2009).

In conclusion, extended infusion has merit from a pharmacodynamic perspective, but comparative trials are needed to demonstrate its validity.

Dosing

The optimal dose for continuous infusion has not been defined well, as no formal dose finding studies have been performed. In clinical practice, two approaches are commonly being used: the first and most simple approach is to administer the same total dose during continuous infusion as commonly administered during intermittent infusion. In the second approach, pharmacodynamic considerations are being applied. This implies that the free fraction should be maintained at a level of four times the MIC of the (suspected) micro-organism causing the infection, concurring with the pharmacodynamic properties of beta-lactams as described: the maximum kill rate is reached at concentrations not higher than four times the MIC. In this scenario, the goal is to administer a dose that will provide a continuous target concentration at four times the MIC. To facilitate the calculation of the daily dose in clinical practice, a previously published normogram can be used. This normogram is based on the above-described target concentration concept (Mouton and Vinks 1996) and shown in Fig. 10.5. The daily dose during continuous administration is directly read from the graph with the total body clearance (TBC) of the drug and the MIC value of the target micro-organism as input. There is a linear correlation between the total body clearance and the creatinine clearance. For the beta-lactams, except for ceftriaxone and cefotaxime, the estimated creatinine clearance can be used as approximation of the total body clearance. To calculate the creatinine

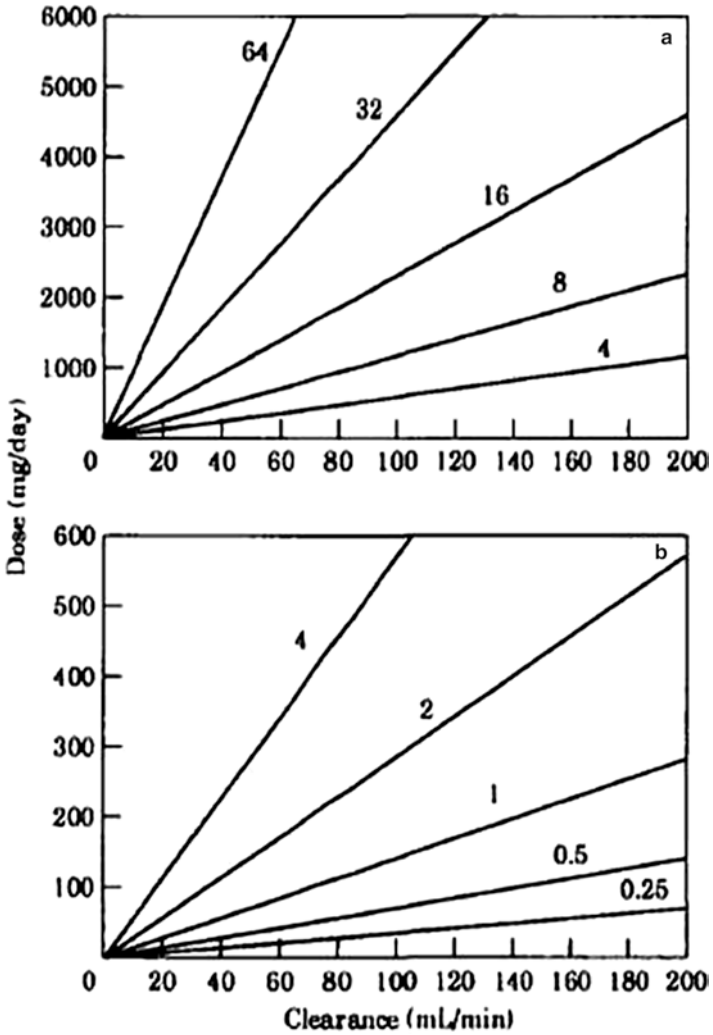

Fig. 10.5 Nomogram for dose adjustments during continuous infusion. When the total body clearance and the target concentrations are known, the total daily dose, given as a continuous infusion can be read from the *y*-axis (Mouton and Vinks 1996)

clearance in adults, the Cockcroft–Gault formula is frequently used (Gault and Cockcroft 1975):

$$CL_{man}\left(mL/\min\right)=\left[140-age\left(years\right)\right]\times\left[weight\left(kg\right)\right]\times 1.23/\left[serum\,creatinine\left(\mu mol/\mathrm{L}\right)\right]$$

$$CL_{woman}\left(mL/\min\right)=CL_{men}\left(mL/\min\right)\times 0.85$$

Or, to convert serum creatinine from μmol/L to mg/dL divide by 88.4

$$CL_{man}\left(mL/\min\right)=\left[140-age\left(years\right)\right]\times\left[weight\left(kg\right)\right]/\left[72\times serum\,creatinine\left(mg/dL\right)\right]$$

$$CL_{woman}\left(mL/\min\right)=CL_{men}\left(mL/\min\right)\times 0.85$$

Alternatively, the daily dose can be determined directly from the following formula:

$$Daily\ \ dose\left(mg\right)=24\left(\mathrm{h}\right)\times TBC\left(\mathrm{L}/\mathrm{h}\right)\times target\ \ concentration\left(mg/\mathrm{L}\right)$$

Since it takes considerable time for concentrations to reach the target concentrations after starting continuous infusion, a loading dose is required. The loading dose can easily be determined as follows:

$$Loading\ \ dose\left(mg\right)=Target\,concentration\left(mg/\mathrm{L}\right)\times volume\ of\ distribution\left(\mathrm{L}\right)$$

For most beta-lactams the volume of distribution of healthy volunteers is approximately 0.2–0.3 L/kg bodyweight. However, hypoalbuminaemia—frequently occurring in ICU patients—is likely to increase the apparent total volume of distribution (Vd) and clearance (CL) of a drug due to lower protein binding, which could translate to lower antibacterial exposures that might compromise the attainment of pharmacodynamic targets, especially for time-dependent antibacterials (Ulldemolins et al. 2011). Low serum albumin levels are very common in critically ill patients, with reported incidences as high as 40–50 % (Ulldemolins et al. 2011). On the other hand, the free fraction of the antimicrobial may be higher in hypoalbuminaemia, which for drugs with a relatively high protein binding may actually yield a net benefit.

Another group of patients with known increased Vd is patients with burn wounds. Aztreonam administered to healthy adults has a Vd of 0.16 ± 0.02 L/kg, whereas the Vd in burn patients has been demonstrated to be 0.31 ± 0.08 L/kg (Friedrich et al. 1991). For imipenem, higher values for Vd were also reported in burn patients compared to healthy adults (Dailly et al. 2003).

For truly individualised antimicrobial therapy, comprehensive PK/PD model-based approaches using clinical software have been described (Vinks 2002; van Lent-Evers et al. 1999). Key PK/PD index estimates and their variability obtained from population analysis are programmed as a specific population model describing absorption, distribution and elimination of the drug in relation to patient-specific parameters such as age, weight, disease state and renal function. Patient demographic and clinical data together with desired target concentrations are entered into the specific sections of the program. Next, a model-based loading dose and maintenance regimen required to optimally achieve the target concentrations is generated. This regimen is administered to the patient and subsequent concentration measurement(s), clinical laboratory data and clinical responses are used as feedback to update the initial model and design a new dosing regimen, if necessary. The latest methods for updating of Bayesian models use the Interacting Multiple Model

(IMM) estimation algorithm, which is currently a popular algorithm in the aerospace community for tracking manoeuvring targets (Bayard and Jelliffe 2004).

Special Situations or Patient Populations

In special patient populations, appropriate antibiotic dosing might not be straightforward and special circumstances may need extra attention in order to optimise therapy. These patients may develop pathophysiological changes that alter the PK of the prescribed antibiotics. Dosing that does not account for these changes may lead to inadequate concentrations and therapeutic failure. To ensure that PK/PD targets are reached, a different approach may be needed compared to patients without these special circumstances.

Critically Ill Patients

Many physiological changes take place in critically ill patients that influence the PK of prescribed antibiotics. Due to sepsis and/or extensive amounts of fluids administered to these patients, the volume of distribution is enlarged and therefore antibiotic concentrations can be decreased. Renal clearance is often also changed in these patients. Therefore, standard dosing regimen might not be optimal in this category of patients.

Studies of several beta-lactams showed that higher antibiotic levels were reached after continuous infusion and were more likely to reach adequate PK/PD targets (Hanes et al. 2000; Georges et al. 2005) in critically ill patients. Both in plasma and in tissues, higher concentrations were reached using continuous infusion compared to intermittent dosing regimen. However, the extent of differences in $\%fT>MIC$ reached using the different regimens was largely depended on the MIC of the micro-organisms causing the disease. The difference in $\%fT>MIC$ is limited between intermittent, extended and continuous infusions for susceptible micro-organisms (Thalhammer et al. 1999; Roberts et al. 2009a).

Most studies comparing the clinical efficacy of continuous infusion to intermittent dosing regimens did not show significant differences in the outcome measures, such as duration of mechanical ventilation, decrease in C-reactive protein and radiological improvement. Roberts et al. (2009b) and Mohd-Hafiz et al. (2011) concluded after systematic review of the data that there is insufficient power in the studies to detect advantages in favour of both strategies. Nevertheless, some studies in critically ill or ICU patients showed significant differences between the two treatment options in favour of continuous infusion. For ceftriaxone, continuous infusion was better compared to intermittent dosing in patients with low APACHE II scores and in patients who were a priori evaluable (Roberts et al. 2007). For piperacillin, there was a temporarily positive effect on the APACHE II score with continuous infusion (Rafati et al. 2006). In several studies, there is a trend toward better outcome after continuous infusion; however, differences with intermittent dosing are often not

statistically significant, primarily because of a low power in these studies. Recently, Dulhunty et al. (2013) showed that in ICU patients clinical cure was significantly better for continuous infusion compared to intermittent bolus dosing (P=0.037); Nevertheless, ICU-free days and hospital survival did not show a significant favourable effect for continuous infusion. It is therefore unlikely to be advantageous for all hospitalised patient populations but is likely to be beneficial for specific groups such as critically ill patients of patients infected with less-susceptible micro-organisms.

Cystic Fibrosis

In CF patients with pulmonary exacerbations and chronic colonisation with *P. aeruginosa*, ceftazidime combined with tobramycin is often used. Several studies compared the efficacy of ceftazidime when administered by continuous infusion compared to a thrice-daily dosing regimen. Tobramycin is given once daily, because the killing effect of aminoglycosides is depended on the C_{max}/MIC and the activity of tobramycin would therefore not benefit from continuous infusion.

PK studies indicate that dosing regimens using continuous infusions ceftazidime provide a more sustained concentration in plasma at levels above the MIC, while concentrations with intermittent dosing decrease to a level below the MIC at the end of the dosing interval (Riethmueller et al. 2009; Rappaz et al. 2000). Continuous infusion of meropenem in CF patients has been shown to result in adequate concentrations, but attention has to be paid to the stability of the drug.

For piperacillin, the pharmacokinetics during both intermittent dosing as well as continuous dosing in CF patients was shown to be non-linear in one study (Vinks et al. 2003). This non-linearity has important implications for calculating the dose used for continuous infusion. During continuous infusion of the same total daily dose, piperacillin steady-state concentrations were, on average, 40 % (range, 6–69 %) lower than expected based on intermittent infusion pharmacokinetic parameter estimates. The non-linear population pharmacokinetic models developed can be used to design effective continuous infusion dosage regimens for the treatment of patients with CF (Vinks et al. 2003).

Clinical studies used spirometric tests such as FEV1 (volume exhaled during the first second of a forced expiratory manoeuver started from the level of total lung capacity) and FVC (forced vital capacity; total amount of air that can forcibly be blown out after full inspiration) to determine the pulmonary performance in CF patients. There were no statistically significant differences in pulmonary performance between patients treated with ceftazidime by continuous infusion compared to intermittent infusion (Riethmueller et al. 2009; Hubert et al. 2009). However, the improvement of FEV1 was better for patients treated with continuous infusion compared to intermittent infusion when they were colonised with ceftazidime-resistant *P. aeruginosa* strains (Hubert et al. 2009). Furthermore, Hubert et al. (2009) showed that the interval between the antibiotic episodes was larger after continuous infusion than after intermittent infusion ($p = 0.04$).

Perioperative Prophylaxis

The aim of perioperative prophylaxis is to prevent post-operative infections, in particular wound infections. To achieve this, antibiotics have to be administered in such a way that adequate blood concentrations are achieved before skin incision and maintained during the whole surgical procedure until the wound is closed. The maintenance of adequate antibiotics levels is important, because the exact time of the contamination is unknown and may occur during the entire procedure as exogenous contamination and can occur at any time until the wound is closed. Especially, when contamination occurs at the end of the dosing interval, the concentrations might be too low. Therefore, the $\%fT>MIC$ is required to be 100 % rather than 40 % of the dosing interval and therefore the dosing interval may need to be shorter than during treatment (Buijk et al. 2004b).

Several studies compared the plasma concentrations reached using bolus infusions before the induction of anaesthesia and using continuous infusion, the latter often after an initial bolus infusion (Waltrip et al. 2002; Adembri et al. 2010; Buijk et al. 2004a). Based on a PK/PD target of reaching T>MIC of 100 % studies using continuous infusions show superiority to intermittent dosing regimens. Whether this has clinical relevance needs to be investigated in an adequately powered randomised clinical trial.

Outpatient Intravenous Therapy

The main determinant of the efficacy of beta-lactams, $\%fT>MIC$, poses them as candidates for intravenous treatment at home, since intermittent-dose i.v. therapy is impracticable outside the hospital setting. Using a portable i.v. infusion pump (or other delivery devices) for continuous administration at home has been shown to be an alternative treatment.

Antibiotics used for this purpose have to be stable at room temperature (or ideally at body temperature when delivery devices are carried under the clothing), so that the delivery device has to be refilled only once or twice daily. In addition, they have to be effective and safe. The drug stability at body temperature limits for example the home use of ceftazidime in patients with CF. The drug is stable at room temperature, but at 37 °C it is only stable for 8 h. Therefore, portable pumps with ceftazidime should be refilled every 8 h or are not to be worn under clothing or during warm summer days (Viaene et al. 2002).

A few studies have been performed in patients with bone or joint infections. Penicillin G administered continuously was found to be feasible for the home-based treatment of a variety of deep-seated infections (Walton et al. 2007). Zeller et al. (2009) studied cefazolin in 100 patients with bone or joint infections. To avoid adverse effect, the concentrations were monitored. In 47 patients, the dose was adjusted, of whom in 38 cases the dose was lowered, resulting in a percentage of

adverse effects of 4.6 %. Eighty-eight patients had a follow-up of at least 12 months with 93 % no signs of infections at follow-up. There were five patients with a relapse with the same susceptible strain, all *S. aureus*. No obvious cause of failure could be identified. Treatment with cefazolin was often combined with gentamicin or rifampicin. The IDSA guidelines state that for continuous dosing with beta-lactams, a complete blood count as well as renal function tests should be performed once a week. For treatment with oxacillin, nafcillin and carbapenems, it is advised to additionally check the liver enzymes weekly (Tice et al. 2004). In summary, continuous administration of beta-lactams was at least as effective as intermittent dosing, also in outpatient setting (Zeller et al. 2009; Howden and Richards 2001; Bernard et al. 2001). Furthermore, it is convenient for the patients and it is safe (Zeller et al. 2009; Bernard et al. 2001).

Continuous Infusion for the Treatment of Resistant Micro-organisms

Since the susceptibility of micro-organisms to various beta-lactams decreases because of emergence of resistance, the use of extended or continuous infusions is becoming more advantageous for maximising the probability of attaining PK/PD targets correlating with efficacy. For micro-organisms with a low MIC, the PK/PD target is easily achieved, even when the antibiotics are not dosed optimally. For micro-organisms with high MIC values, dosing is more critical. Roberts et al. (2009a) showed the differences in target attainment for various MIC values for both extended and continuous infusion for meropenem. The Monte Carlo simulations (MCS) performed in this study show that for micro-organisms with high MICs (4–16 mg/L) the use of extended or continuous infusion compared to intermittent infusion is likely to be advantageous to achieve the PK/PD target of 40 % *f*TMIC (Fig. 10.6). For example, considering a Gram-negative micro-organisms with an MIC of 8, the probability that the target of 40 % *f*T > MIC will be reached is 68.8 % for intermittent dosing of 2,000 mg q8h, whereas the probability to achieve this target is 96.9 and 100 % for extended and continuous infusion, respectively (Roberts et al. 2009a). The improvement of %*f*T > MIC for higher MICs is also shown using an in vitro pharmacodynamic model for ceftazidime performed with doses chosen to match values obtained in critically ill patients (Alou et al. 2005) analogous to human volunteers (Mouton and den Hollander 1994). Lorente et al. (2009) showed that there is a significant difference in clinical outcome in favour of continuous infusion in patients treated with piperacillin/tazobactam for ventilator-associated pneumonia, but these advantages were only for micro-organisms causing the VAP with an MIC of minimal 8 mg/L (OR = 10.79, 95 % CI 1.01–588.24; $p = 0.049$).

The fact that the difference between intermittent and extended or continuous infusion is most important for micro-organisms with high MICs, might be the cause that most clinical studies did not show significant results between the treatment groups.

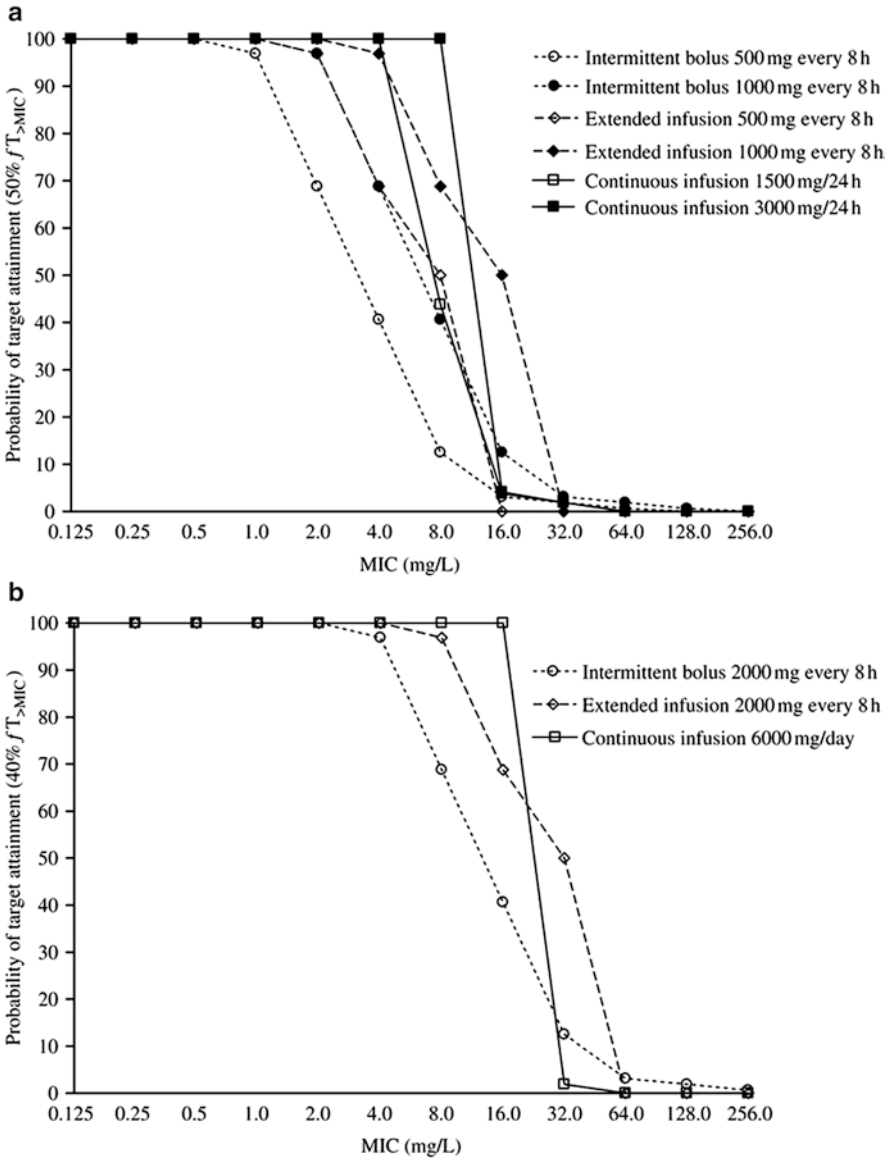

Fig. 10.6 Probability of target attainment for meropenem by intermittent bolus (infused over 3 min), extended infusion (infused over 4 h) or continuous infusion as (**a**) 1,500–3,000 mg per 24 h period and (**b**) 6,000 mg per 24 h period. All patients given continuous infusion doses initially received a 500-mg loading dose. The chosen target for analysis was 40 % $fT>MIC$ for plasma concentrations (Roberts et al. 2009a)

This is supported by data of Hubert et al. (2009) showing that there were no significant results in a randomised cross-over study using ceftazidime in CF patients colonised with *P. aeruginosa*. But, after analysis of the subgroup of patients colonised with resistant strains, there was a significant difference ($p<0.05$) in clinical outcome in favour of continuous infusion. On the other hand, Patel et al. (2009) studied continuous infusion of piperacillin–tazobactam in a retrospective cohort study and found no differences on the 30-day mortality after stratification by MIC value.

Conclusion

Preclinical studies have shown a clear advantage of continuous infusion. Generally, studies comparing the plasma PK of intermittent and continuous administration of beta-lactams have shown that administration by continuous infusion maintains superior concentrations throughout the treatment period and that the PTA is higher. The superiority is less clear in studies comparing clinical outcomes, and a large comparative trial is badly needed. Differences in clinical outcomes are most often documented in analyses of special subgroups, such as critically ill patients or patients with infections caused by resistant micro-organisms.

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Chapter 11
Macrolides and Ketolides

Françoise Van Bambeke

Abstract Macrolides and ketolides are characterized by a very wide tissular distribution, which is related to their capacity to accumulate in the acidic compartments of the cells. This property is considered an advantage, because it concentrates the drug at the site of infection. Yet, the low serum levels consecutive to this tissular distribution may favor the selection of resistance. Macrolides are essentially bacteriostatic and ketolides are slowly bactericidal. The pharmacodynamic indice that best predicts efficacy is the free 24 h-AUC/MIC ratio for both subclasses. Despite their high concentration inside the cells, macrolides and ketolides remain bacteriostatic against intracellular bacteria, with a potency similar to that observed extracellularly. New formulations have been developed to optimize patient's adherence (extended release tablets) or to further increase antibiotic concentration at the site of infection (powders for inhalation).

Keywords Macrolides • Kétolides • AUC/MIC • Tissue distribution

Pharmacokinetic Development of Macrolides and Ketolides and Impact of Chemical Structure on Pharmacokinetic and Pharmacodynamic Properties

Erythromycin, a natural product isolated from *Streptomyces erythreus* (McGuire et al. 1952), was introduced in the clinic in the mid 1950s and remained for long the only large-scale macrolide used. A major limitation of this drug, however, comes

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from its instability in acidic medium, which results in poor and highly variable bioavailability. This instability is due to the simultaneous presence of a keto function (in position 9) and of an hydroxy function (in position 6), which react in acidic medium to generate a spiroketal which is inactive (Fig. 11.1) (Kirst and Sides 1989). A series of macrolides were therefore developed, which showed an improved stability because they are unable to form a spiroketal (Fig. 11.1). These include 14-membered macrolides like erythromycylamine (Massey et al. 1970, 1974), clarithromycin (Fernandes et al. 1986; Morimoto et al. 1984), roxithromycin (Chantot et al. 1986), and the 15-membered azalide azithromycin (Bright et al. 1988; Djokic et al. 1987). 16-membered macrolides [spiramycin (Kellow et al. 1955), josamycin (Nitta et al. 1967), midecamycin (Kanazawa and Kuramata 1976), miocamycin (Kawaharajo et al. 1981; Omoto et al. 1976), and rokitamycin (Sakakibara et al. 1981)] are intrinsically stable because they do not have a keto function in their macrocycle. In ketolides (Bryskier 2000; Van Bambeke et al. 2008), acid stability is obtained by the lack of cladinose, combined with the substitution of the 6-O position as in telithromycin [HMR-3647 (Denis et al. 1999)], cethromycin [ABT-773 (Or et al. 2000)], and solithromycin [CEM-101 (Hwang et al. 2008)], or of the 9-keto function (as in modithromycin [EDP-420 (Wang et al. 2004)]). Beside this pharmacokinetic advantage, the chemical modifications brought to ketolides also improve their antimicrobial activity and favorably modify their pharmacodynamic profile, making them more bactericidal than macrolides at high concentration (Drago et al. 2005; Zhanel et al. 2002). Thus, the heteroalkyl side chain present in all ketolides improves the activity against both macrolide-susceptible and resistant bacteria by allowing for an additional binding to the domain II of the ribosomal subunit, which allows them to keep activity on methylated ribosomes. Moreover, because they lack the cladinose sugar, ketolides do not induce methylase expression and are not recognized by Mef efflux pumps in *S. pneumoniae* (Douthwaite 2001; Douthwaite and Champney 2001; Van Bambeke et al. 2008).

Macrolides and ketolides also share a weak basic character because they all possess an aminated function on their desosamine moiety that is protonable in acid media. This basic character is responsible for their high level of accumulation inside eukaryotic cells. As proposed for cationic amphiphilic drugs (de Duve et al. 1974), macrolides and ketolides can indeed freely diffuse through the membranes in their non-protonated form and are then trapped in the acidic compartments of the cells (lysosomes) in their less diffusible protonated form (Carlier et al. 1987, 1994). Some molecules have an additional aminated function (erythromycylamine, azithromycin). This may contribute to explain the higher cellular accumulation of azithromycin (Carlier et al. 1994).

Pharmacokinetics

General Pharmacokinetic Properties

The main pharmacokinetic properties of macrolides and ketolides are summarized in Table 11.1.

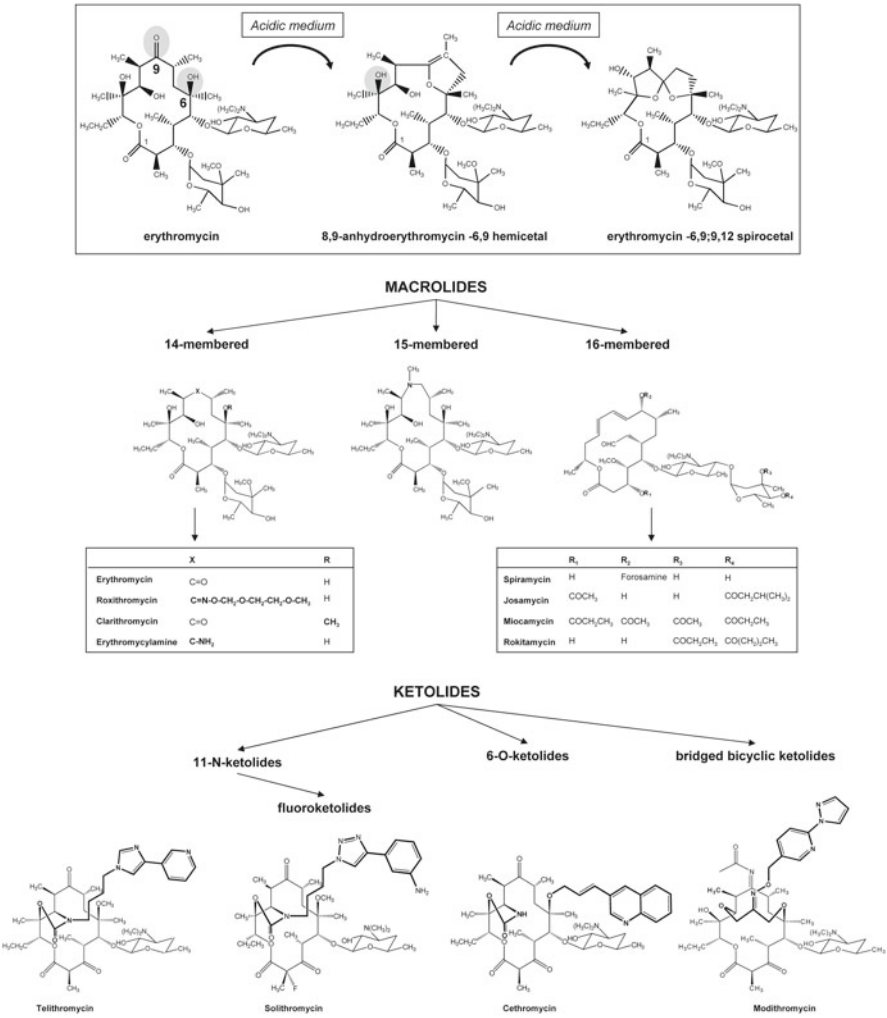

Fig. 11.1 Chemical instability of erythromycin and chemical structure of macrolides and ketolides. Mechanism responsible for the inactivation of erythromycin in acidic medium. The ketone in position 9 reacts with the hydroxyl in position 6 to generate a hemicetal, which reacts again with the hydroxyl in 12 to produce a ketal. Both the hemiketal and the ketal are microbiologically inactive [Adapted from Kirst and Sides (1989)]. Neomacrolides were made acidostable by either removing the 9-keto function and replacing it with another function (roxithromycin, erythromycylamine, azithromycin) or by substituting the 6-hydroxyl group (clarithromycin). 16-membered derivatives are intrinsically stable because of the absence of a ketone function in the cycle. Likewise, acid stability in ketolides is obtained by removing of cladinose combined with the substitution of the 6-O position (as in telithromycin, cethromycin or solithromycin) or of the 9-keto function (as in modithromycin)

Table 11.1 Main pharmacokinetic properties of macrolides and ketolides

Drug	Erythromycin	Clarithromycin	Roxithromycin	Azithromycin	Telithromycin (HMR-3647)	Cethromycin (ABT-773)	Modithromycin (EDP-420)	Solithromycin (CEM-101)
References	Brogden and Peters (1994)	Fraschini et al. (1993), Peters and Clissold (1992)	Puri and Lassman (1987)	Foulds et al. (1990)	Kuehnel et al. (2005), Lippert et al. (2005), Namour et al. (2001), Shi et al. (2005), Traunmuller et al. (2009)	Conte et al. (2004), Lawrence (2001), Pletz et al. (2003)	Jiang et al. (2009)	Still et al. (2011)
Dose for PK studies	500 mg bid po	500 mg po	150 mg bid po	500 mg po	800 mg po	150 mg po	400 mg (1 day followed by 200 mg)	800 mg po (1 day followed by 400 mg)
C_{max} (mg L^{-1})	3	3.4	6.8	0.4	1.9	0.32	0.54	1.3
T_{max} (h)	1.9–4.4	2–3	2	2.5	3		3.25	3.5
T1/2 (h)	2	5.7	8–13	72	7.16	5.7	15.8	6.65
Vd (L kg^{-1})	0.64	3–4			2.9			
Bioavailability (%)	25–60	55	72–85	37	57	60		
Prot. binding (%)	65–90	42–50	73–96	12–40	60–70	85–95		85
Tissue/serum	0.5		1–2	50–1,150	1–5 0.3–0.6			
AUC (mg h L^{-1})	4.4–14	46	70	2–3.4	8.25	1.6	14	14
Conventional dosage in adults	500 mg 4×/day	250–1,000 mg 2×/day	150 mg 2×/day	500 mg 1×/day or 500 mg on day 1 and 250 mg on days 2–5	800 mg 1×/day	300 mg 1×/day[a]		800 mg po on day 1 and 400 mg on days 2–5[a]
Conventional dosage in children	12.5 mg/kg 4×/day	7.5 mg/kg 2×/day	3 mg/kg 2×/day	10 mg kg^{-1} on day 1 and 5 mg kg−1 on days 2–5				

[a]Based on ongoing clinical trials

Absorption

Due to their amphiphilic character, macrolide and ketolide antibiotics are capable of diffusing through membranes, and are therefore in general well absorbed by oral route, with the maximum concentration reached within 2–3 h. The effect of food intake on absorption depends on the formulation, with capsules and powdered suspensions of azithromycin and erythromycin (base or stearate) being best absorbed when taken 1 h before or 2 h after meals (Zhanel et al. 2001). In most cases, digestive tolerance is improved when the drug is taken with food.

Distribution

The most striking pharmacokinetic property of macrolides and ketolides is their large volume of distribution (Bahal and Nahata 1992; Zeitlinger et al. 2009), which is related to their ability to accumulate inside eucaryotic cells.

In humans, macrolides and ketolides distribute largely in most tissues, where they reach concentrations that are well above serum concentrations, in keeping with their capacity to accumulate in cells. However, their penetration in the CNS is limited (Kearney and Aweeka 1999), and only subtherapeutic levels can be reached in this compartment. Penetration in epithelial lining fluid and in alveolar macrophages is best documented (Table 11.2). Additional data on penetration in other tissues are nevertheless available for azithromycin and telithromycin. For azithromycin, sustained and high concentrations are also found in the lung (Di Paolo et al. 2002), tonsils (Foulds et al. 1991), and prostate (Foulds et al. 1990) as well as in inflamed blister fluid (Freeman et al. 1994). Telithromycin achieves high and prolonged concentrations in the lung (Kadota et al. 2002; Khair et al. 2001), nasal mucosa and ethmoid bone (Kuehnel et al. 2005), tonsils (Gehanno et al. 2003), female genital tract (Mikamo et al. 2003), and inflamed blister fluid (Namour et al. 2002). Its free concentration in soft tissues (subcutis and muscle) is close to the free serum concentration (Gattringer et al. 2004; Traunmuller et al. 2009).

The consequence of this large distribution is that serum levels are relatively low (see Table 11.1), so that pharmacodynamic indices may be difficult to reach in the central compartment (see this chapter's section on pharmacodynamics). However, the fact that their tissular and cellular concentrations are high may be an advantage for the treatment of infections localized in these compartments (Schentag and Ballow 1991; Zhanel et al. 2001). The slow release of macrolides out of the cells is indeed suggested to allow for the progressive release of antibiotic at the site of infection (Gladue et al. 1989; Hand and Hand 2001; McDonald and Pruul 1991), with white blood cells playing the role of shuttle for the drug (Amsden et al. 1999). On the other hand, their prolonged retention inside the cells is thought to be a major determinant for their activity against intracellular pathogens (Gladue et al. 1989; McDonald and Pruul 1991; Pascual et al. 2001). This concept, however, will need to be revisited in the light of pharmacodynamics (see section on intracellular pharmacodynamics).

Table 11.2 Distribution of macrolides and ketolides in the respiratory tract

	AUC (mg h L^{-1})				
Antibiotic (dose)	Alveolar macrophages	Ratio to serum	ELF[a]	Ratio to serum	Reference
Clarithromycin (200 mg)	4,840	190	390	3.5–15	Kikuchi et al. (2008) and calculated based on the data of Rodvold et al. (1997)
Clarithromycin extended release (1,000 mgl)	5,730	205	179	6.4	Gotfried et al. (2003)
Azithromycin (500 mg)	1,674	540	7.7	2.5	Lucchi et al. (2008)
Azithromycin extended release (2,000 mg)	7,028	703	17	1.7	Lucchi et al. (2008)
Telithromycin (800 mg)	5,060	425	184	15	Calculated based on the data of Muller-Serieys et al. (2001)
Cethromycin (300 mg)	636	180	24	6.5	Conte et al. (2004)
Solithromycin (400 mg)	1,500	180	80	10	Rodvold et al. (2012)
Modithromycin (400 mg)	2,560	245	212	21	Furuie et al. (2010)

[a]Epithelial lining fluid

Elimination

Macrolides and ketolides are metabolized through the cytochrome P450 (CYP) 3A subfamily, and are also moderate to potent inhibitors of the CYP3A4 pathway, causing numerous drug–drug interactions (Pai et al. 2006; Shakeri-Nejad and Stahlmann 2006). They are thereafter eliminated via the bile with the exception of clarithromycin, which shows significant elimination in the urine (Fraschini et al. 1993). Erythromycin shows the shorter and azithromycin the longer half-life, which is correlated with their differential cell retention. These differences have important consequences in terms of number of daily administrations (Table 11.1) and treatment duration in order to optimize pharmacodynamic indices (see section on intracellular pharmacodynamics).

Cellular Pharmacokinetics

The accumulation of macrolides and ketolides has been mainly studied in phagocytic cells [macrophages or polymorphonuclear neutrophils (PMN)]. Variable cellular concentrations (see Table 11.3) have been reported, which can be easily explained by

Table 11.3 Cellular accumulation (cellular to extracellular concentration ratio) [a] of macrolides and ketolides as reported in in vitro studies

	Cell type			
Antibiotic	Macrophages	PMN	Epithelial cells/ fibroblasts	References
Erythromycin	4–38	8	6–12	Bosnar et al. (2005), Carlier et al. (1987), Montenez et al. (1999), Villa et al. (1988)
Clarithromycin	16			Mor et al. (1994)
Roxithromycin	25–60	14	8–23	Carlier et al. (1987), Montenez et al. (1999), Villa et al. (1988)
Azithromycin	40–160	20–517	10–85	Blais et al. (1994), Bosnar et al. (2005), Carlier et al. (1994), Hand and Hand (2001), Lemaire et al. (2009), Mandell and Coleman (2001), Montenez et al. (1999), Pascual et al. (1997)
Telithromycin	5–71	31–300	8	Bosnar et al. (2005), Lemaire et al. (2009), Mandell and Coleman (2001), Pascual et al. (2001), Vazifeh et al. (1998)
Cethromycin	12	207–500	30	Bosnar et al. (2005), Garcia et al. (2003), Labro et al. (2004)
Solithromycin	370			Lemaire et al. (2009)

[a]Extreme values when multiple studies have been published

the differences in models and experimental conditions used (concentration range and incubation time). Generally speaking, however, azithromycin and ketolides accumulate to the highest levels, probably related to the dicationic character of azithromycin on the one side and to the greater lipophilicity of ketolides on the other side. These drugs distribute mainly in lysosomes, with a smaller proportion found in the cytosol (Carlier et al. 1987, 1994; Labro et al. 2004; Togami et al. 2010b; Villa et al. 1988). Influx transporters have been suggested to play a role in the uptake of ketolides in white blood cells (Labro et al. 2004; Togami et al. 2010b; Vazifeh et al. 1998), but the kinetics of their accumulation and their subcellular distribution are fully coherent with a passive mechanism of diffusion–segregation. Efflux from the cells is usually slow, but it can be facilitated by the activity of the multidrug transporter P-glycoprotein (Munic et al. 2010; Pachot et al. 2003; Seral et al. 2003b).

Pharmacodynamics

Antibiotics are categorized as either concentration- or time-dependent drugs. Macrolides were for long considered as time-dependent antibiotics, with an efficacy related to the time interval during which their concentration at the infected site remains above the MIC of the offending organism (Carbon 1998; Craig 1998). This was suggested based on the fact that their action on bacteria is essentially bacteriostatic, and that their activity can only be maintained as long as the antibiotic remains bound to the ribosome (this is similar to what is observed with beta-lactams, but is in sharp contrast with aminoglycosides which also impair protein synthesis but also cause translation mistakes [and, therefore, lethal events] in direct correlation to their concentration). Yet, macrolides show post-antibiotic effects (time necessary to observe bacterial regrowth upon drug withdrawal) spanning between one to several hours (Dornbusch et al. 1999; Odenholt et al. 2001), in relation to their particular pharmacokinetic profile, suggesting that time of exposure may not be the only driver for efficacy.

Studies in murine pneumonia models showed indeed that not only time during which clarithromycin concentration remains above the MIC but also the ratio of the area under the concentration–time curve from 0 to 24 h ($AUC_{0\text{-}24h}$) to the MIC and the C_{max}/MIC were significantly correlated to antibacterial efficacy, median survival time, and total percent survival (Tessier et al. 2002). Further animal studies (Ambrose et al. 2007; Craig et al. 2002; Tessier et al. 2005) confirmed that the free AUC to MIC ratio is the major PK/PD determinant for the activity of both macrolides and ketolides.

In Vitro Pharmacodynamic Studies

In Vitro Pharmacodynamic Models

All macrolides are essentially bacteriostatic compounds, causing no or minimal decrease in colony forming units (CFU) (Drago et al. 2005; Furneri and Nicoletti 1991). Ketolides prove slightly more efficient against gram-positive organisms, causing a 1–4 log decrease in CFU of *S. aureus, S. pneumoniae, or S. pyogenes* over 24 h (Barcia-Macay et al. 2006; Drago et al. 2005; Kays et al. 2007; Woosley et al. 2010). Their killing activity develops over time but is also concentration dependent; it is influenced by the bacterial inoculum (Boswell et al. 1998). Both macrolides and ketolides display post-antibiotic effects that vary between 1 and 8 h (Boswell et al. 1998; Odenholt-Tornqvist et al. 1995); which is suggested to allow long dosing interval despite low serum concentrations. Yet, these low concentrations still leave open the question of the risk of facilitating the selection of resistant organisms.

In vitro pharmacodynamic models have evaluated the efficacy of macrolides and ketolides in conditions that mimic exposure in human serum or tissues after

treatment with conventional doses. For clarithromycin, this type of study suggested that a bactericidal effect against *S. pneumoniae* could be achieved as soon as time above the MIC was ≥90 % or the area under the curve to MIC ≥61 h; a static effect, or even a regrowth, was observed when these values fell to 8 % and 17.3 h. These pharmacodynamic indices are easily reached in epithelial lining fluid than in serum, which may explain the microbiological success observed in the treatment of pneumonia for isolates with MIC as high as 8 mg L^{-1} (Noreddin et al. 2002). Roxitromycin was less effective than azithromycin when simulating their respective pharmacokinetics in tonsils. Regrowth was observed after 6 h against *S. pneumoniae* and 26 h against *S. pyogenes* with roxithromycin, while viable counts reached the limit of detection in 8–10 h with azithromycin, with no regrowth within 48 h (Firsov et al. 2002). Likewise, simulated free azithromycin concentrations in serum, epithelial lining fluid, and middle ear fluid allow to maintain the concentration above the MIC during 100 % of the time, and an area under the curve to MIC ratio ≥36.7 h against macrolide-susceptible *S. pneumoniae,* resulting in a bactericidal effect (Zhanel et al. 2003). Yet, insufficient coverage was obtained against resistant strains (Zhanel et al. 2003), as well as against gram-negative bacteria like *H. influenzae* or *M. catharralis* (Treyaprasert et al. 2007). For telithromycin, a bactericidal effect was observed when simulated concentrations in serum and epithelial lining allowed to reach a C_{max}/MIC ≥3.5 and an area under the curve to MIC ≥25 h, but a bacteriostatic effect was observed when these exposures were twice lower. This means that telithromycin at its conventional dosage should be able to eradicate streptococci with an MIC of 0.25 mg L^{-1} in serum and 1 mg L^{-1} in epithelial lining fluid (Zhanel et al. 2005). This type of approach also led to the conclusion that at human-simulated exposure, telithromycin can achieve higher AUC/MIC ratios than clarithromycin against *S. pneumoniae*, and therefore higher chances of microbiological eradication, while the contrary holds true for *S. aureus* (Alferova et al. 2005). Fewer data are available for the other ketolides. Cethromycin was shown to be bactericidal, even against macrolide-resistant strains (Neuhauser et al. 2003). Modithromycin activity is AUC/MIC dependent, as the other ketolides, with simulated values of approximatey 10 and 16–20 h required to reach a maximal effect against *H. influenzae* and *S. pneumoniae,* respectively (Homma et al. 2010). The latter value is thus of the same order of magnitude as what has been reported for telithromycin.

Intracellular Pharmacodynamics

Because of their high level of accumulation inside eucaryotic cells, macrolides are claimed to be active against intracellular pathogens. They are, indeed, active in vitro against numerous bacteria causing intracellular infections, like *Legionella, Chlamydia* (Blackman et al. 1977; Horwitz and Silverstein 1983), or *Mycobacteria* (Wildfeuer and Haberreiter 1997). However, in vitro models comparing them with other antibiotic classes suggest that their intracellular activity is rather limited, because of (a) their bacteriostatic character and (b) the defeating effect on

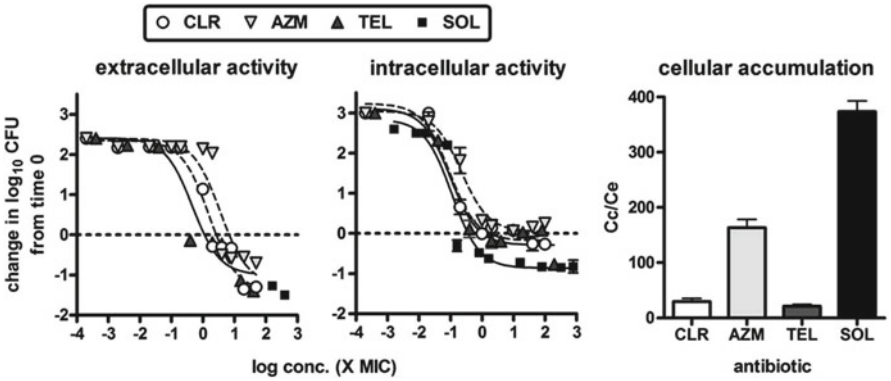

Fig. 11.2 Comparison of the extracellular and intracellular activity of macrolides and ketolides against *S. aureus* ATCC25923 and of their cellular accumulation in a model of THP-1 human monocytic cells. Activity was evaluated after 24 h of incubation in broth (*left panel*) or in infected cells (*middle panel*) with each antibiotic, using a wide range of extracellular concentrations spanning from 0.0001× and 1,000× its MIC (the *dotted line* corresponds to a bacteriostatic effect). Cellular accumulation was measured after 24 h of incubation of non-infected cells with 10 mg L^{-1} of each drug (*CLR* clarithromycin, *AZM* azithromycin, *TEL* telithromycin, *SOL* solithromycin). One can see that despite high levels of cellular accumulation, macrolides and ketolides are less effective against intracellular than against extracellular *S. aureus*, with only solithromycin being able to reach a −1 log intracellular effect. Likewise, potencies (evaluated by the static concentrations, i.e. the concentrations for which there is no change form the initial inoculum) are of the same order of magnitude against extracellular and intracellular bacteria, with no clear correlation with the respective level of accumulation of each drug. Adapted from Lemaire et al. (2009)

their intrinsic activity of the acidic pH prevailing in lysosomes (see Fig. 11.2 for an illustration). In-depth studies following the influence of time or of concentration on intracellular activity show indeed that azithromycin was only able to prevent the intracellular growth of bacteria sojourning in the cytosol like *L. monocytogenes* or in vacuoles like *S. aureus* and to cause a minor (<1 log) reduction in the intracellular counts of *L. pnemophila* (Barcia-Macay et al. 2006; Carryn et al. 2002; Lemaire et al. 2009). The importance of cellular concentration for activity is further illustrated by the fact that inhibitors P-glycoprotein allow to reach this maximal effect upon exposure to lower extracellular concentrations, by increasing the antibiotic concentration in the infected compartment (Seral et al. 2003a, b). A ketolide like solithromycin systematically showed an increased maximal efficacy (1–1.5 log decrease), but this was not the case for telithromycin, at least against *S. aureus* (Lemaire et al. 2009). It therefore appears that other parameters than accumulation and distribution need to be taken into account in the intracellular activity of antibiotics, among which the expression of activity in the intracellular environment, the bacterial responsiveness, and the cooperation with cell defense mechanisms probably play a central role (Carryn et al. 2003; Van Bambeke et al. 2006).

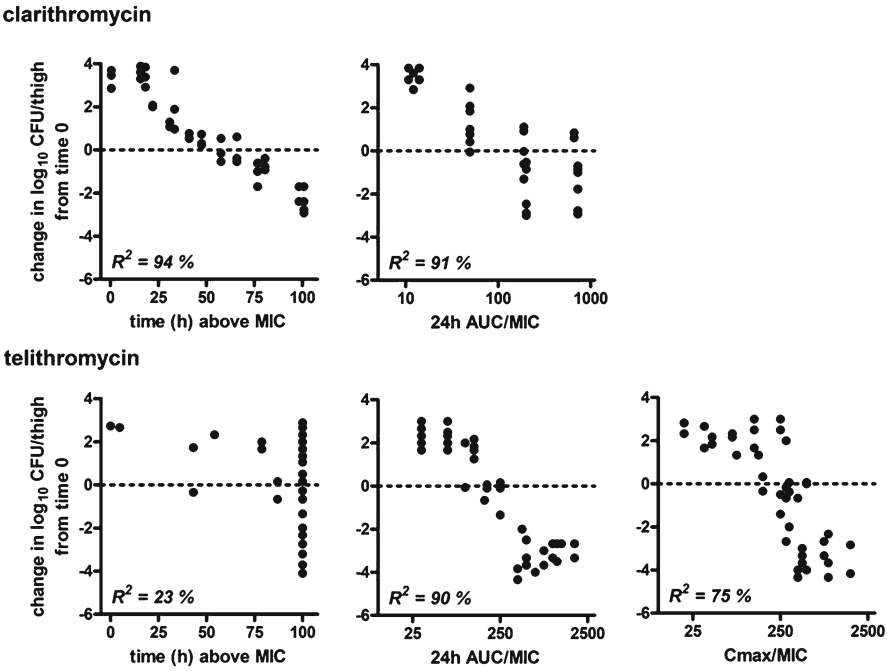

Fig. 11.3 Correlation between efficacy of clarithromycin (*upper panel*) or telithromycin (*lower panel*) against *S. pneumoniae* ATCC10813 and PK/PD parameters in the neutropenic mouse model. The *graphs* show that the efficacy of clarithromycin correlates with AUC/MIC and time above MIC, while that of telithromycin correlates with AUC/MIC and to a lower extent C_{max}/MIC. Adapted from Craig et al. (2002) and Vesga et al. (1997)

Animal Models

Early studies suggested that macrolides were time-dependent antibiotics (Carbon 1998; Craig 1998). This concept has been revised over the last 10 years, so that it is now accepted that the parameter determining efficacy in vivo is AUC/MIC for both macrolides and ketolides (See Fig. 11.3).

Tessier and coworkers were the first to suggest an interdependency between time above the MIC, AUC/MIC, and C_{max}/MIC ratio when studying the activity of clarithromycin in a model of murine pneumonia (Tessier et al. 2002) and came thus to the conclusion that AUC/MIC ratio is the best predictor of efficacy. Almost at the same time, Craig and coworkers refined this concept by correlating efficacy to the free AUC/MIC ratio, with a value of 20–35 h being needed to reach a static effect for both macrolides and ketolides in a model of pneumonia in neutropenic mice (Craig et al. 2002). Under these conditions, static effects can still be observed with strains showing low level of resistance (efflux-mediated resistance mainly) (Hoffman et al. 2003; Noreddin et al. 2002). Tissular penetration was also

recognized as a major determinant in efficacy, since drugs with longer tissular half-life appeared more effective in a model of pneumonia in leucopenic mice (Veber et al. 1993). Infiltration of inflamed tissues by phagocytes could further help increase local concentration of macrolides (Girard et al. 1990; Schentag and Ballow 1991), but the acidic pH of most abscesses is deleterious to their activity.

Tessier and coworkers demonstrated later that free AUC/MIC ratio was predictive of telithromycin efficacy in the same pneumonia model, with stasis observed for values ranging between 20 and 100 h and maximal effect for values >200 h. In similar experiments, the free AUC/MIC ratio was confirmed to be the main determinant of efficacy for cethromycin, with static effect reached at a value of 50 h (Kim et al. 2002). For solithromycin, stasis was obtained with an AUC/MIC ratio of about 1.4 h for the free fraction in the serum or the total drug in the ELF (Andes et al. 2010).

In vivo pharmacodynamic studies of macrolide activity against intracellular bacteria confirm their poor efficacy, with azithromycin causing a 0.2 log drop in intracellular counts in a model of *S. aureus* peritonitis (Sandberg et al. 2009). This goes thus against the idea that intracellular breakpoints could be higher because of the high accumulation of these drugs (Amsden 2001).

Human Pharmacodynamics

Pharmacodynamics of macrolides and ketolides have also been examined in humans, with the aim of determining target attainments rates and for rationalizing dosages of currently used molecules or establishing those of molecules in development.

For registered drugs, Noreddin and coworkers showed that, upon treatment with conventional dosages, the probability of attainment of a free AUC/MIC_{90} target of 30 h in serum or ELF was systematically higher for telithromycin (99 % in serum; 100 % in ELF) than for clarithromycin (91.3 % in serum, 99.9 % in ELF) and even more than for azithromycin (81.3 % in serum, 82.3 % in ELF) against susceptible pneumococci (Noreddin et al. 2009). For telithrmoycin, Lodise and coworkers proposed that a fAUC/MIC ratio of 3.375 h in serum and of 27 h in ELF can predict microbiological eradication (Lodise et al. 2005). They attribute these low values to the high local concentration of the drug at the site of infection and/or its delivery from PMN migrating to the site of infection. In pharmacodynamic studies examining other ketolides vs *S. pneumoniae,* Conte and coworkers reported that treatment with 150 or 300 mg cethromycin allows to reach an AUC/MIC_{90} of approximately 110 and 340 h, respectively (Conte et al. 2004), which is well above the proposed target of 50 (Kim et al. 2002). Furuie and coworkers reported an AUC/MIC_{90} of 84 h in patients having received 400 mg modithromycin (Furuie et al. 2010), but no target value has been proposed for this drug so far. With respect to solithromycin, recent data suggest that at dose of 800 mg at day one followed by a daily dose of 400 mg allows to reach the target of ELF AUC/MIC >1.3 h for stasis (Andes et al. 2010) with a probability of 99.9 % for MICs as high a 1 mg L^{-1} (Okusanya et al. 2010).

Table 11.4 PK/PD target for macrolides and ketolides and corresponding breakpoints

Antibiotic	PK/PD target	fAUC (h)	PK/PD bkpt (mg L^{-1})	CLSI bkpt (S≤; mg L^{-1})	EUCAST bkpt (S≤; mg L^{-1})	Reference for PK/PD target
Clarithromycin	fAUC/MIC > 20–30 h	~23	~0.8	0.25	0.25	Tessier et al. (2002)
Roxithromycin	fAUC/MIC > 20–30 h	~7	~0.25		0.5	
Azithromycin	fAUC/MIC > 20–30 h	~2	~0.07	0.5	0.25	Tessier et al. (2002)
Telithromycin	fAUC/MIC > 3.375 h	~2.5	~0.75	1	0.25	Lodise et al. (2005)
Cethromycin	AUC/MIC > 50 h corresponding to a fAUC/MIC of ~ 5 h	~1.6	~0.03	NA	NA	Kim et al. (2002)
Solithromycin	fAUC/MIC > 1 h	~2	2	NA	NA	Andes et al. (2010)

Table 11.4 shows the proposed PK/PD targets for these compounds and compares the PK/PD breakpoints that can be calculated on this basis with the susceptibility breakpoints from CLSI and EUCAST. One can see that the current susceptibility breakpoints are of the same order of magnitude as the PK/PD breakpoints, suggesting they correctly take into account pharmacodynamic criteria.

New Formulations

Extended Release

In spite of the already long half-life of macrolides, extended release formulations have been developed by pharmaceutical companies in order to obtain appropriate AUCs while at the same time reducing the number of daily administrations. Figure 11.4 and Table 11.2 compare the pharmacokinetic properties of these formulations with those of the corresponding immediate release formulation. The extended release formulation of clarithromycin allows giving the daily dose in a single administration, with almost no change in pharmacokinetic parameters as far as AUC is concerned (Gotfried et al. 2003; Guay et al. 2001). The serum concentration remains longer above the susceptibility breakpoint and sustained levels are obtained in epithelial lining fluid and macrophages.

An extended release form of azithromycin has also been registered. Because of the extended half-life of this drug, this formulation allows for a single dose treatment.

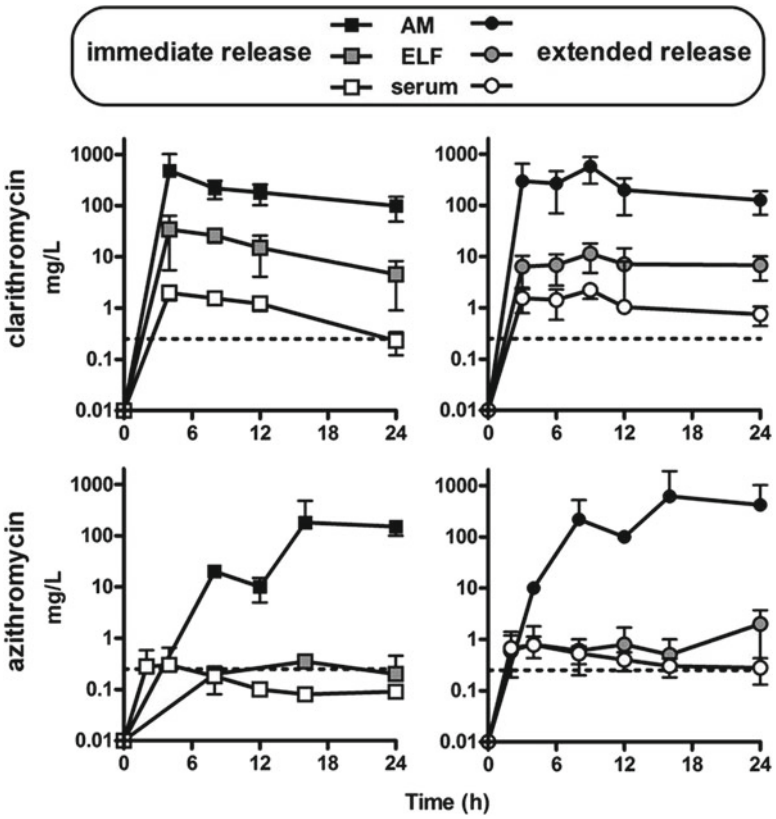

Fig. 11.4 Comparative pharmacokinetics of clarithromycin and azithromycin with immediate release and extended release formulations in serum, epithelial lining fluid (ELF), and alveolar macrophages (AM). For clarithromycin (*upper panel*), volunteers received nine doses of 500 mg immediate release form every 12 h or five doses of 1,000 mg extended release form; pharmacokinetics was evaluated after the last dose [constructed based on data from Gotfried et al. (2003), Rodvold et al. (1997)]. For azithromycin (*lower panel*), volunteers received a single dose of 500 mg immediate release form or of 2,000 mg extended release form [constructed based on data from Lucchi et al. (2008)]. The *dotted horizontal line* corresponds to the EUCAST susceptibility breakpoint of each drug (0.25 mg L^{-1})

The formulation, which has been developed using the microsphere technology, increases the serum AUC from 3.1 mg h L^{-1} to 10 mg h L^{-1}, which is not negligible in view of the low serum concentrations of this drug (Lucchi et al. 2008). It also maintains the serum concentration above the susceptibility breakpoint for 24 h and increases the exposure to the drug in ELF as well as inside macrophages (Lucchi et al. 2008) or in sinuses (Ehnhage et al. 2008; Fang et al. 2009). Of interest also, the overall exposure ($AUC_{0-120\,h}$) is similar or even slightly higher in serum or in white blood cells after administration of a single dose of extended release formulation vs. a 3 days treatment with the 500 mg immediate release form; C_{min} at 120 h is similar

with the two dosage regimens as well (Liu et al. 2007). As for the immediate release formulation, efficacy best correlates with the AUC/MIC ratio, with significantly higher success rates observed when this ration is >5 (Muto et al. 2011). It should be kept in mind, however, that the dose administered is 2 g instead of 500 mg for the immediate release formulation, but no difference in tolerability between the two formulations has been reported so far (Lucchi et al. 2008). This formulation may thus offer an opportunity of optimizing patient adherence (Swainston and Keam 2007).

Aerosols

Beside their indications in respiratory tract infections, macrolides are also widely used in cystic fibrosis or bronchiolitis where they have shown their potential in improving respiratory function through their immuno-modulatory and anti-inflammatory effects (Shinkai et al. 2008). It is therefore not surprising that aerosol formulations of macrolides are now being developed. Azithromycin dry powder inhalers (Zhang et al. 2010) have been evaluated in rats. The best formulation allows to deliver high concentrations in the respiratory tracts with an AUC in the ELF that is 161-fold higher than that obtained with a same dose administered by IV route and a bioavailability of 43 %. Likewise, telithromycin aerosols are also investigated, but rather for the treatment of pulmonary infections (Togami et al. 2010a), with again higher concentrations in lung epithelial lining fluid and alveolar macrophages and lower concentrations in serum than following the administration of an oral formulation.

Conclusion

The pharmacokinetic profile of macrolides and ketolides is essentially characterized by their wide tissular distribution due to their accumulation in the lysosomal compartment of the cells. This however, does not necessarily translate in high efficacy against intracellular bacteria because of the bacteriostatic (or slowly bactericidal for ketolides) character of their activity and of the deleterious effect of acid pH on their activity. Pharmacodynamic studies have shown that the free AUC/MIC ration is the best predictor of efficacy. Yet, the high volume of distribution of these drugs also translates in low serum concentrations and therefore low AUC in the central compartment. PK/PD breakpoints take however this limitation into account and clearly define the conditions for rationally using these drugs.

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Chapter 12
Glycopeptides

Inge C. Gyssens

Abstract Glycopeptides are a class of antibiotic drugs that is composed of glycosylated cyclic or polycyclic nonribosomal peptides. Older molecules used in clinical practice are vancomycin and teicoplanin. Oritavancin, dalbavancin, and telavancin belong to the subclass of lipoglycopeptides. Glycopeptides inhibit bacterial cell wall peptoglycan synthesis of aerobic and anaerobic Gram-positive bacteria. Glyco (lipo)peptides are not absorbed orally and have to be administered intravenously. In this chapter, the pharmacokinetics (Pk) and the pharmacodynamics (Pd) of the glycopeptides are studied. Pk in serum as well as protein binding and elimination are reviewed. Pharmacodynamic data include MICs of Gram-positive bacteria, PK/Pd effects in in vitro systems, animal models and human studies. Adverse effects of glycopeptides on the host are concentration related nephro- and ototoxicity. In the past, because of fear of toxicity, the older glycopeptides have been underdosed in many settings. The implementation of Pk/Pd knowledge into clinical practice by e.g. administering higher doses of vancomycin and teicoplanin and using continuous infusion of vancomycin is urgently needed. This chapter contains clinical vignettes showing the benefit of Therapeutic Drug Monitoring of glycopeptides.

Keywords Lipoglycopeptides • Telavancin • Therapeutic drug monitoring

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General Description

Glycopeptides are a class of antibiotic drugs that is composed of glycosylated cyclic or polycyclic nonribosomal peptides. Vancomycin, isolated from *Amycolatopsis orientalis* found in soil (McCormick et al. 1956), was the first member of this new class (Pfeiffer 1981). Teicoplanin has the ability to anchor onto the binding sites on the growing cell wall; this effect is primarily due to the presence of hydrophobic lipophilic side chains and results in increased potency (Van Bambeke 2004). After 30 years of vancomycin use, the increased frequency of vancomycin-resistant strains has prompted the development of new antibiotics for the treatment of infections caused by Gram-positive bacteria. Newer glycopeptides are oritavancin, dalbavancin, and telavancin belong to the subclass of lipoglycopeptides. They are structurally different from vancomycin and teicoplanin and have increased potency and less potential for development of resistant organisms. Oritavancin and dalbavancin are still in development and will not be discussed in this chapter. Telavancin is approved in the USA and Canada (2009) for the treatment of adults with complicated skin and skin structure infections (cSSSI) caused by susceptible organisms. Recently (September 2011), telavancin was approved in all member states of the European community, Norway, and Iceland, for the treatment of adults with nosocomial pneumonia (NP), including ventilator-associated pneumonia, known or suspected to be caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Telavancin is also active against vancomycin-resistant organisms.

Glycopeptides inhibit bacterial cell wall peptidoglycan synthesis of aerobic and anaerobic Gram-positive bacteria. They have a similar spectrum. They bind to the amino acids within the cell wall preventing the addition of new units to peptidoglycan. In particular, they bind to acyl-D-alanyl-D-alanine in peptidoglycan. Unlike vancomycin, which demonstrates a slow, primarily bacteriostatic profile, the newer lipoglycopeptides show rapidly bactericidal properties. Unlike other glycopeptides, telavancin also disrupts cell membrane barrier function by a noncovalent interaction between its lipophilic side chain and the lipid bilayer of the bacterial cell membrane (Lunde et al. 2009). This ultimately causes the disruption of the cell membrane integrity and increased membrane permeability, resulting in cell lysis (King et al. 2004; Higgins et al. 2005).

Glycopeptides have large molecular masses: vancomycin 1,450, teicoplanin 1,993, and telavancin 1,755, respectively.

Pharmacokinetics

Bioavailability

Glyco (lipo)peptides are not absorbed orally.

After oral vancomycin administration, only very low serum concentrations are obtained, except in the presence of renal disease and bowel inflammation.

Low serum concentrations have been measured in these patients with renal failure in whom vancomycin elimination is decreased. Spitzer and Eliopoulos (1984) noted serum concentrations between 11.4 and 20.3 μg/mL in a hemodialysis patient with antibiotic-associated colitis given 500 mg orally four times daily.

For teicoplanin and vancomycin, the oral route can be used to treat pseudomembranous colitis and Clostridium difficile-associated diarrhea (CDAD). Telavancin is only available in intravenous formulation.

Protein Binding

The binding of vancomycin to protein ranges from 10 to 50 % (Ackerman et al. 1988; Albrecht et al. 1991). Teicoplanin is predominantly (90 %) bound to plasma proteins (albumin) and binding appears linear with rising concentrations (Bernareggi et al. 1991). In an in vitro study, it appeared that this high degree of serum protein binding impaired the bactericidal activity of teicoplanin, compared with that of vancomycin (Bailey et al. 1991), but Dykhuisen et al. (1995) reported that in vivo in volunteers the serum bactericidal activity of teicoplanin was not impaired by its high degree of protein binding. In another in vitro study, coagulase-negative staphylococci, multiplying within a clot of human plasma, were shown to be partly protected from inhibition and killing by both vancomycin and teicoplanin. This effect, however, was more pronounced for teicoplanin (Cunningham et al. 1997). Serum albumin level plays a major role in the variability of the unbound fraction of teicoplanin. The impact of lower serum albumin levels on teicoplanin pharmacokinetics was assessed by studying the relationship between total and free concentrations of teicoplanin in serum samples obtained from patients receiving teicoplanin therapy for Gram-positive bacterial infections. In addition, the contribution of serum albumin concentrations to the unbound fraction of teicoplanin was determined. One hundred ninety-eight serum samples were obtained from 65 patients undergoing routine therapeutic drug monitoring of teicoplanin. Free serum teicoplanin was separated by ultrafiltration, and total and free serum concentrations of teicoplanin were determined by a fluorescence polarization immunoassay. Regression analysis was then performed to build a prediction model for the free serum teicoplanin concentration from the total serum teicoplanin concentration and the serum albumin level using the first 132 samples. The predictive performance of this model was then tested using the next 66 samples. Free serum teicoplanin concentrations (Cf) (μg/mL) were predicted using a simple model constructed using total serum teicoplanin (Ct) (μg/mL) and albumin concentrations (ALB) (g/dL): $Cf = Ct/(1 + 1.78 \times ALB)$. This model could estimate free serum teicoplanin concentrations with a small bias and an acceptable error. The measured free concentration of teicoplanin will lie between 0.63 and 1.38 times the predicted concentration in 95 % of cases (Yano et al. 2007).

Telavancin is also highly (93 %) bound to serum proteins. The clinical relevance of this high level of protein binding is not fully clear (Leuthner et al. 2006; Tsuji et al. 2008). In the study by Leuthner et al., telavancin MICs in the presence of

serum increased on average twofold. Despite the increase in MIC, this effect had no impact on bactericidal activity as demonstrated by kill curves at concentrations of 4× MIC or greater. This may be due to a weaker protein binding association constant than predicted by protein binding experiments (Shaw et al. 2005). Alternatively, the effect on bacterial membrane integrity may be less affected by protein binding, as suggested by Hegde et al. (2004). The concentration-dependent activity of telavancin was apparent both in the presence and absence of serum (Leuthner et al. 2006). Another study evaluated the activity of telavancin, daptomycin, vancomycin, and teicoplanin in the presence of albumin and human and mouse serum (Tsuji et al. 2008) and observed telavancin and daptomycin to have more activity than the free fraction drug concentration previously reported.

Pharmacokinetic Profiles in Serum

PK of Vancomycin

Pharmacokinetic parameters are shown in Table 12.1. The pharmacokinetic profile of i.v. vancomycin is complex and can be characterized by either a two- or three-compartment pharmacokinetic profile (Matzke et al. 1984; Rodvold et al. 1988; Rotschafer et al. 1982). In patients with normal creatinine clearance, vancomycin has a distribution phase of approximately 30 min to 1 h and an elimination half-life of 6–12 h. The volume of distribution is 0.4–1 L/kg (Matzke et al. 1986; Rodvold et al. 1988). In adult volunteers, serum concentrations 1 h after a 500-mg i.v. dose are 13–22 μg/mL, and after a 1-g dose, 25–40 μg/mL (Blouin et al. 1982; Healy et al. 1987; Boeckh et al. 1988). Peak serum levels with a dose of 1 g every 12 h are usually between 25 and 40 μg/mL, and trough levels are between 5 and 10 μg/mL. In adults with normal renal function, Healy et al. (1987) reported little intersubject variation in serum concentrations—a mean level of 33.7 μg/mL (SD 3.8 μg/mL, range 26.5–40.5 μg/mL) 1 h after a 1-g dose. Most of the variation could be accounted for by differences in weight of the subjects. There is more variation in the elimination half-life, which ranges from 3 to 13 h, the mean being approximately 6 h (Rotschafer et al. 1982). Healy et al. (1987) demonstrated that some vancomycin accumulation occurred in their normal subjects with repeated dosing. Trough levels increased from 5.4 to 11.2 μg/mL after five doses of 500 mg given 6-hourly and from 4.9 to 7.9 μg/mL after three i.v. 12-hourly doses of 1 g. Continuous infusion of 30 mg/kg over 24 h in 13 patients resulted in a plateau level of 24 ± 6 μg/mL (Wysocki et al. 1995).

Children

Preterm infants do not have fully mature renal systems, and vancomycin clearance is impaired as a result. The prolonged elimination half-life in preterm infants may be related to immaturity of the kidneys, and to a lesser extent possibly to immaturity

Table 12.1 Pharmacokinetics of (lipo) glycopeptides in adults

Parameter	Vancomycin	Teicoplanin (6 mg/kg)	Telavancin (10 mg/kg)
Volume of distribution (Vd) (L/kg)	0.4–1	0.9–1.6 (steady state)	0.13 (steady state)
Peak (C_{max}) μg/mL	25–40 (1 g dose)	43	108 70 (10 mg/kg dose)[a]
$AUC_{0-24\,h}$ (mg h/L)	260 (15 mg/kg dose)	600	780
Total serum clearance (Cl_s) mL/min	62.7	12.2	13.1
Metabolism	Very low metabolization, metabolites inactive	Some liver metabolism. Two metabolites with some activity identified in urine	No metabolites were detected in vitro studies
Elimination	Virtually entirely eliminated by the kidneys	83 % of dose excreted unchanged by the kidneys	Primarily eliminated by the kidneys
Half-life ($t_{1/2}$) hours	3–13	88–182	8
Protein binding	10–50 %	>90 %	~90 %
Bioavailability	Not absorbed orally	Not absorbed orally	Not absorbed orally

Vancomycin (Matzke et al. 1984, 1986; Healy et al. 1987; Blouin et al. 1982; Boeckh et al. 1988; Rodvold et al. 1988; Gyssens 2010a), teicoplanin (Gyssens 2010b), telavancin: telavancin EMA product information (European_Medicines_Agency 2011)
[a]Rubinstein et al. (2011)

of the liver. Actual dosage recommendations are based on postconceptional age and weight and generally result in peak levels of 30–40 μg/mL and trough levels of 5–10 μg/mL (Naqvi et al. 1986; James et al. 1987). In a retrospective population pharmacokinetic study, 108 newborns with suspected central line-related septicemia during the first month of life received 30 mg/kg per day vancomycin divided into two doses, regardless of gestational or postconceptional age. Targets were a trough concentration between 5 and 15 μg/mL and a peak <40 μg/mL. Of the 108 patients, 34.3 % of measured trough concentrations and 17.6 % of peak concentrations were outside the desired therapeutic range. The model that best fitted the data included clearance and volume/kg and was independent of gestational age. Simulation showed that a dosing schedule of 30 mg/kg per day, irrespective of gestational age, in three doses was optimal. The optimal scheme was then tested prospectively in 22 patients. Mean trough concentrations before the second dose were 8.2 ± 2.2 μg/mL versus a predicted trough of 8.9 ± 2.5 μg/mL. No peak levels >40 μg/mL were found. With this dosing scheme, the authors concluded that there is no need for routine monitoring of peak serum concentrations (de Hoog et al. 2000, 2004).

Schaad et al. (1980) also studied vancomycin concentrations in older infants and children. A 15 mg/kg dose given to seven term infants produced a mean peak level of 29.8 μg/mL, and in infants aged 1–12 months, 10 and 15 mg/kg doses resulted in peak levels of 26.1 and 28.0 μg/mL, respectively. Similar levels were noted in children

3–5 years of age given these two doses. Calculated elimination half-lives were 5.9–9.8 h for newborn infants, 4.1 h for older infants, and 2.2–3.0 h for children. Serum level monitoring on 11 other patients showed there was no evidence of accumulation with repeated doses.

Renal Impairment

Patients with impaired renal function have a great variability in vancomycin half-life. Therefore, maintenance doses of vancomycin should be guided by serum levels. Vancomycin accumulates in these patients and a modified dosage schedule with serum level monitoring is necessary. In renal failure, the vancomycin half-life is variable and may be greatly prolonged up to 17 days (Matzke et al. 1984). Because vancomycin clearance from the serum is linearly related to creatinine clearance, a variety of methods have been developed for determining vancomycin dosage adjustment in patients with impaired renal function. In the nomogram compiled by Moellering et al. (1981), the total daily dose/kg is adjusted according to the creatinine clearance value. The latter can be estimated, if it cannot be measured directly, by the Cockroft–Gault equation, taking into account the patient's age, sex, and serum creatinine value. Matzke et al. (1984) prepared a nomogram for patients with impaired renal function in which, after an initial loading dose of 25 mg/kg, the vancomycin dose remains constant at 19 mg/kg, but the dosage interval depends on the creatinine clearance. This nomogram can be used for initiation of vancomycin therapy for functionally anephric patients on hemodialysis, but not for patients treated with intermittent or continuous peritoneal dialysis (see below). Rodvold et al. (1988), on the basis of a detailed PK study in 37 patients with varying degrees of renal impairment, developed the following equation to calculate vancomycin doses: dose (mg/kg per 24 h) = 0.227 × creatinine clearance (mL/min) + 5.67. Very little vancomycin is removed from the body by hemodialysis (Lindholm and Murray 1966; Eykyn et al. 1970). In 29 anephric patients managed by hemodialysis at 3-day intervals, a single 1-g i.v. dose of vancomycin given over a period of 30 min resulted in a mean peak serum level of 48.3 μg/mL, which declined to 15 μg/mL within 3–5 h, but was still 3.5 μg/mL after 18 days; the mean elimination half-life was 7.5 days (Cunha et al. 1981). A study by Quale et al. (1992) suggested that use of newer dialysis membranes with greater permeability to larger molecules (high-flux membranes) altered vancomycin pharmacokinetics. Vancomycin levels posthigh-flux membrane dialysis (HFHD) were only 63 % of predialysis levels, with low vancomycin levels in the dialysate suggesting binding of vancomycin to the membrane. However, rebound in vancomycin levels occurs after completion of HFHD, so different dosing regimens are probably not required for these patients (Pollard et al. 1994). Traditionally, patients undergoing hemodialysis were given an i.v. loading dose of 1 g or 15 mg/kg. A serum level was then measured 5–7 days later, and the vancomycin dose repeated when the level fell below 5–10 μg/mL. This strategy often results in underdosing, as is illustrated in a recent study. A single dose of vancomycin 35 mg/kg administered during HFHD in oliguric patients with

end-stage renal disease did not achieve the therapeutic serum concentration necessary for once weekly dosing. Patients on long-term HFHD in the outpatient setting received vancomycin 35 mg/kg, rounded to the nearest 250 mg, administered during hemodialysis at a rate of 1 g/h via an infusion pump. No patient achieved a vancomycin concentration of ≥10 μg/mL on study day 8 (mean serum concentration, 5.1 μg/mL). Six patients (83 %) who received vancomycin predialysis had undetectable vancomycin levels (<3.5 μg/mL) by study day 8 ($n=6$). Patients who received vancomycin postdialysis maintained a mean serum concentration of 6.4 μg/mL at day 8 ($n=3$) (Crawford et al. 2008). Peritoneal dialysis also results in minimal clearance of vancomycin (Moellering 1984). Magera et al. (1983) did not find any appreciable change in vancomycin concentrations before, during, or after completion of chronic intermittent peritoneal dialysis, and serum levels were maintained above 4 μg/mL for 8 days after a single 1-g i.v. dose. Morse et al. (1987) studied four patients undergoing CAPD who received a 15 mg/kg i.v. dose. The mean peak serum vancomycin concentration was 57.1 μg/mL, at 24 h the level was 19.8 μg/mL, and 7 days later the level was still 8.6 μg/mL. The mean terminal elimination half-life was 111 h. The mean dialysate concentration at the end of the initial dwell was 5.8 μg/mL, and subsequent end-dwell dialysate concentrations were greater than 2 μg/mL for most exchanges over a 1-week period. For patients receiving CAPD, vancomycin doses of 15 mg/kg every 7 days (Krothapalli et al. 1983), or 23 mg/kg initially followed by 17 mg/kg every 7 days (Blevins et al. 1984), have been recommended, but should be guided by serum levels. In contrast to peritoneal and hemodialysis, some vancomycin is cleared by patients undergoing hemofiltration (Matzke et al. 1986). Continuous hemodiafiltration removes larger amounts of vancomycin (Bellomo et al. 1990), and twice-daily administration of 7.5 mg/kg i.v. was suggested by Santre et al. (1993). However, because of patient-to-patient variability resulting from factors such as blood flow rates through the filtration apparatus, monitoring of serum concentrations is necessary to guide dosing.

Impaired Hepatic Function

Renal mechanisms account for almost all vancomycin elimination, but vancomycin can be detected in feces and bile, indicating that some hepatic clearance also occurs. Brown et al. (1983) found that the vancomycin elimination half-life was prolonged in cancer patients with abnormal liver function, but Rodvold et al. (1988) could not correlate abnormalities in liver function with changes in vancomycin clearance. Dosage adjustment is probably not necessary in patients with liver impairment, but monitoring of serum levels would be prudent.

Pregnancy

Some patient groups may require higher dosages because of increases in volume of distribution or renal clearance. In pregnancy, Salzman et al. (1987) reported that a

dose of 57 mg/kg was required to maintain recommended drug levels in a patient who was 30 weeks pregnant. Monitoring of serum concentrations is therefore required.

Obesity

In obese patients, pharmacokinetic parameters, such as volume of distribution and elimination half-life, are significantly different from those of patients at or near their normal body weight (Vance-Bryan et al. 1993). A dose of 0.5 g every 6 h or 1 g every 12 h will generally produce suboptimal peak and trough concentrations, so dosing based on the absolute body weight, not the ideal body weight, should be used to calculate initial vancomycin doses in these patients (Moellering 1984).

Critically Ill Patients

Intensive care patients also require higher doses. A retrospective pharmacokinetic analysis of serum levels obtained in routine vancomycin monitoring was performed in 46 vancomycin-treated intensive care unit (ICU) patients over 18 years old not needing renal replacement support. Population analyses were made by the standard two-stage approach. Vancomycin clearance and distribution volume were estimated individually assuming a one-compartment pharmacokinetic model. Pharmacokinetic and pharmacodynamic analysis was performed by Monte Carlo simulation. In the ICU patients, higher Vd (nearly twice the quoted value of 0.72 L/kg) and different vancomycin clearance–creatinine clearance relationship were found. Renal function, the APACHE score, age, and serum albumin accounted for more than 65 % of drug clearance variability. Vancomycin standard dosages led to a 33 % risk of not achieving the recommended $AUC_{24\ h}$/MIC breakpoint for *S. aureus* (del Mar Fernandez de Gatta Garcia et al. 2007). Another study was conducted in intensive care patients to evaluate retrospectively the importance of a Bayesian pharmacokinetic approach for predicting vancomycin concentrations to individualize its dosing regimen in 18 critically ill patients admitted to intensive care units following cardiothoracic surgery. The possible influence of some coadministered drugs with important hemodynamic effects (dopamine, dobutamine, and frusemide) on vancomycin pharmacokinetics was assessed. Vancomycin dosage regimens predicted by the Bayesian method [D(a)] were compared retrospectively with Moellering's nomogram-based dosages [D(M)] to assess possible major differences in vancomycin dosing. In 8 out of 18 patients, much higher dosages were required despite no major difference in attained vancomycin steady-state trough concentration or estimated creatinine clearance. In four patients, the withdrawal of cotreatment with hemodynamically active drugs was followed by a sudden substantial increase in the vancomycin C_{min} steady state, despite no major change in body weight or estimated creatinine clearance being observed. The authors highlighted the risk of possible subtherapeutic serum vancomycin concentrations when these drugs are coadministered and the need for therapeutic drug monitoring of vancomycin in these situations (Pea et al. 2000).

Cancer Patients

In a comparative study of infants and children with and without malignancy, vancomycin clearance rates and dosage requirements were higher in the cancer patients (Chang 1995). On the basis of this and another study (Chang et al. 1994), a starting dose of 10 mg/kg six times daily was suggested for children with cancer, with further dosing guided by serum vancomycin estimations. Vancomycin clearance was also higher in 35 adult patients with hematologic malignancy, although dosage requirements were not greatly increased (Fernandez de Gatta et al. 1993). The mechanism of the increased vancomycin clearance is unknown.

PK of Teicoplanin

The pharmacokinetics of teicoplanin follow a triexponential decay, the alpha, beta, and gamma half-lives being 0.4–1 h, 4.7–15.4 h, and 83–168 h, respectively. The kinetics of distribution is not dependent on dose. Teicoplanin's volumes of distribution are 0.07–0.11 (initial phase), 1.3–1.5 (distribution phase), and 0.9–1.6 (steady state) L/kg, the value being greater in studies with longer sampling periods. Studies before 1990 used shorter sampling times and so reported lower values for the Vd. Teicoplanin has a long serum half-life (88–182 h) which reflects the slow distribution in the tissues.

Single-dose pharmacokinetic studies were carried out on six volunteers (Verbist et al. 1984) doses of 3 and 6 mg/kg were given i.v. over 5 min, or a dose of 3 mg/kg was given by i.m. injection. Immediately after i.v. injection, the mean peak values after the 3 and 6 mg/kg doses were 53.48 and 111.81 μg/mL, respectively; these levels fell rapidly in the first 8 h, but at 24 h they still exceeded 2 and 4 μg/mL, respectively. After the i.m. injection of 3 mg/kg, a mean peak serum level of 7.12 μg/mL was reached in 2 h and thereafter serum levels followed those attained after an identical i.v. dose, being greater than 2 μg/mL at 24 h. The elimination half-life of the drug was about 47 h. Similar results were obtained by Buniva et al. (1988), who gave 400 mg of teicoplanin i.v. over 60 s to volunteers. Plasma concentrations averaged 71.7 μg/mL at 5 min after administration, decreasing to 4.0 μg/mL at 24 h (Buniva et al. 1988). After a 200-mg i.m. dose, the mean peak serum level in eight volunteers was 7 μg/mL after 4 h; when this dose was repeated every 12 h, this peak rose to 12 μg/mL after the sixth dose. When the drug was continued in a dose of 200 mg daily, trough serum levels were 5.4–7.3 μg/mL from day 2 to day 6 (Williams and Gruneberg 1984). Single doses of 15, 20, and 25 mg of teicoplanin per kg of body weight to five adult volunteers by a 30-min i.v. infusion resulted in peak levels at the end of the infusion averaging 194, 197, and 253 μg/mL, respectively (Del Favero et al. 1987). Mean concentrations in plasma 24 h after administration were 10.5, 13.6, and 19.8 μg/mL, respectively. Terminal half-lives averaged 88, 83, and 92 h.

Based on early pharmacokinetic studies, it was suggested that for clinical dosing regimens of teicoplanin, dosing every 12 h for approximately 48 h should be used, followed by once-daily dosing thereafter (Outman et al. 1990). After 1990,

multiple-dose administration has been reexamined. In a randomized crossover study in 10 healthy volunteers, two loading doses of 6 or 12 mg/kg at 12-h intervals were given on day 1 followed by 6 or 12 mg/kg every 24 h for 13 days. After 14 days, an estimated 93 % of the steady state was achieved. Total body clearance did not change significantly with increasing dose (Thompson et al. 1992). In a randomized double-blind study, three different regimens were assessed in 4–10 healthy volunteers (3, 12 and 30 mg/kg/day for 14 days). Steady-state concentrations increased in proportion to dosage. After 12 mg/kg IV, serum concentrations were between 10 and 100 μg/mL, reaching 15–20 μg/mL at 12 h and 10 μg/mL at 24 h. The total body clearance was between 10.5 and 13.4 mL/h/kg and renal clearance was 8.5–11.6 mL/h/kg. The $t_{1/2}$ gamma varied between 59 and 231 h. Even with the higher dose of 30 mg/kg, no dose-related differences were found in total or renal clearance. However, the V(d)ss fell with the increasing dose. There was a trend to decrease in the $t_{1/2}$ gamma. Saturable tissue binding is probably responsible because the total body clearance is unaffected by dose (Smithers et al. 1992; Wilson 2000).

A randomized study compared teicoplanin concentrations following alternate or daily i.v. dosing in healthy adults. Trough serum concentrations were compared following administration of 12 mg/kg of body weight every 12 h for three doses and then 15 mg/kg every 48 h for four doses ($n=16$ subjects) or 6 mg/kg every 12 h for two doses and then 6 mg/kg every 24 h for nine doses ($n=8$ subjects). The mean ± standard deviation trough concentrations in serum on day 11 (24 and 48 h after administration of the last dose for the daily and alternate-day dosing schedules, respectively) were 16.0 ± 2.1 and 17.9 ± 3.5 μg/mL for subjects receiving the two regimens, respectively. Throughout the study the individual trough concentrations in serum in the alternate-day dosing group constantly exceeded 10 μg/mL (Rouveix et al. 2004).

Children

The pharmacokinetics of teicoplanin were assessed after a single dose and under multidose conditions in 12 infants and children. Study patients ranged in age from 2.4 to 11 years. Each patient received teicoplanin 6 mg/kg body weight given i.v. over 20–30 min, once daily for 5 consecutive days. Multiple timed blood and urine samples were obtained over the 6-day sampling period and were analyzed by both microbiological assay and HPLC. Three-compartment pharmacokinetic analysis was used to describe the drug's disposition characteristics. Peak and 24 h trough serum teicoplanin concentrations averaged 39.3 and 1.8 μg/mL after the first dose with little accumulation observed after 5 days of therapy. Teicoplanin disposition was variable; V(d)ss ranged from 0.31 to 0.68 L/kg, $t_{1/2}$ gamma from 6.5 to 18.1 h, and CI from 29 to 51 mL/h/kg. A substantial amount of the administered drug distributed rapidly to the largest, third compartment, with egress approximately four-fold slower than ingress. The majority of the drug was excreted unchanged in the urine. Teicoplanin administration was well tolerated by all study subjects. Using the teicoplanin pharmacokinetic data derived in the study, it was estimated that a dose

of teicoplanin 8 mg/kg body weight administered every 12 h should achieve target serum trough concentrations averaging 11 μg/mL in children. Higher doses, e.g., 15 mg teicoplanin/kg administered every 12 h, may be needed for the treatment of deep-seated staphylococcal infections and/or endocarditis (Reed et al. 1997).

Twenty-one critically ill children aged between 7 days and 12 years were treated with teicoplanin (three loading dosages of 10 mg/kg at 12 h intervals, followed by a maintenance dosage of 10 mg/kg/day). Serum teicoplanin concentrations were monitored by HPLC. Mean concentrations in plasma 30 min after drug administration were 20 ± 16.1 μg/mL. The volume of distribution was 0.30 L/kg and the terminal half-life was 17.41 h. Only 11 % of trough values were >10 μg/mL (established as target). It was concluded that in critically ill children a dosage of 10 mg/kg/day does not assure serum trough values >10 μg/mL (Sanchez et al. 1999).

Renal Impairment

The administration of a 5 and 10 mg/kg dose of teicoplanin to seven anuric patients immediately after the end of hemodialysis gave mean C_{max} of 62.80 and 122.43 μg/mL, mean AUC of 526.43 and 1103.98 mg h/L, mean half-life ($t_{1/2}$) of 109.09 and 107.06 h, mean clearance rates of 12.85 and 12.44 mL/min, mean apparent volumes of distribution of 1.68 and 1.68 L/kg, and mean volumes of distribution at steady state of 0.31 and 0.28 L/kg, respectively. Trough serum levels above 10 μg/mL were found for 24 h after the administration of the 5 mg/kg dose and for 48 h after the administration of the 10 mg/kg dose. Teicoplanin was not detected in the dialysate. Its concentrations in both the arterial and the venous lines of the fistulae were similar (Papaioannou et al. 2002). One single dose of 10 mg/kg teicoplanin was administered intravenously to eight anuric patients undergoing CAPD. Blood and dialysate were sampled at regular time intervals for 48 h postdrug infusion. Concentrations of teicoplanin were determined by microbiological assay. Teicoplanin serum levels >10 μg/mL were detected for 24 h after administration. All dialysate concentrations were very low. Teicoplanin presented two phases of elimination: an early first phase and a late second phase. Mean C_{max} was 75.56 μg/mL, mean half-life of the early elimination phase was 3.34 h, mean half-life of the late elimination phase was 61.68 h, mean AUC was 1491.92 mg h/L, mean clearance rate was 10.68 mL/min, mean apparent Vd was 0.80 L/kg, and mean $V_d$ss was 0.22 L/kg. Mean dialysate excretion was 3.16 % and mean peritoneal clearance rate was 0.023 mL/min (Stamatiadis et al. 2003).

Critically Ill

In a study in critically ill patients, serum was collected frequently during day 0 and then pre- and 1 h postdose on days 1, 2, 3, 5, 7, and every third day thereafter during treatment. The teicoplanin PK profile was best described by a two-compartment model ($n = 26$). The clearance was 4.97 ± 1.58 L/h. Serum levels exceeded 4 μg/mL

for the entire dosing interval in all subjects (400 mg dose every 12 h) with an AUC/MIC of 399.3 (95 % CI 329.6–469.0). However, only 4 of 14 exceeded trough serum concentrations of 10 μg/mL (Whitehouse et al. 2005).

Teicoplanin is concentrated in phagocytes where it appears to aid the killing of ingested organisms such as *S. aureus* (Carlone et al. 1989).

PK of Telavancin

At an infusion of 30–120 min, telavancin demonstrates linear and predictable pharmacokinetics within a dosage range of 7.5–15 mg/kg (Shaw et al. 2005; Wong et al. 2008). In the phase I preclinical trial, steady state was achieved by day 3 or 4 without evidence of accumulation. Maximum concentrations (C_{max}) and AUC were highest after a 30-min infusion. The serum half-life is 8.0 ± 1.5 h (single dose) and 8.1 ± 1.5 h (multiple doses) (Shaw et al. 2005; Wong et al. 2008, 2009). Pharmacokinetic parameters are summarized in Table 12.1.

The tissue penetration of telavancin is extensive, reaching common sites of infection. A human blister study observed a favorable mean penetration in blister fluid (40 %) compared with plasma when telavancin was dosed at 7.5 mg/kg intravenously every 24 h for 3 days in nine healthy volunteers (Sun et al. 2006). Telavancin achieved a mean AUC of 241 ± 33 μg/mL h in blister fluid versus 604 ± 83 μg/mL h in plasma.

When intrapulmonary distribution of telavancin 10 mg/kg/day for 3 successive days was observed with the use of bronchoalveolar lavage in 20 healthy subjects, telavancin penetration into epithelial lining fluid (ELF) and alveolar macrophages was found to be substantial (Gotfried et al. 2008). Telavancin produced a mean AUC_{ELF}/AUC_{plasma} penetration ratio of 10 % (74.8 ± 73.2 vs. 740.4 ± 125.2 mg/L h) (Lodise et al. 2008a).

Telavancin is taken up by eukaryotic cells and localizes in lysosomes, causing mild morphological alterations without evidence of lipid metabolism alterations (Barcia-Macay et al. 2008). Concentrations of telavancin in alveolar macrophages were reportedly higher than ELF concentrations, with concentrations at 24 h of 42.0 ± 31.4 in alveolar macrophages versus 0.89 ± 1.03 μg/mL in ELF (Gotfried et al. 2008). This corresponds to a mean concentration of telavancin in ELF and alveolar macrophages of approximately 8–85-fold its MIC_{90} (0.5 μg/mL) for MRSA. Moreover, unlike daptomycin, telavancin activity did not appear to be affected by the presence of pulmonary surfactant (Gotfried et al. 2008).

Elderly

In a phase I, open-label, single-dose, sex-stratified study the safety and tolerability, and the effect of sex on the pharmacokinetic disposition, of a single i.v. dose of telavancin 10 mg/kg was performed in healthy elderly (≥65 years) subjects, eight

men and eight women (mean ± SD ages 70.6 ± 6.1 and 70.8 ± 5.5 years, respectively). Each subject received a 60-min i.v. infusion of telavancin 10 mg/kg. Telavancin plasma concentrations were determined by liquid chromatography with tandem mass spectrometric detection at regular intervals up to 48 h after the start of the infusion. Pharmacokinetic parameters of telavancin were determined by noncompartmental analysis. The telavancin plasma concentration–time curves and pharmacokinetic parameters for both sexes were comparable. Pooled mean ± SD clearance, half-life, and volume of distribution at the steady state were 12.2 ± 1.4 mL/h/kg, 9.3 ± 1.3 h, and 156 ± 12 mL/kg, respectively. The pooled mean ± SD plasma concentration of telavancin 24 h postdose was 10.8 ± 1.6 μg/mL, exceeding the telavancin MIC_{90} for key Gram-positive pathogens (0.5 μg/mL). Ten (63 %) of the 16 subjects reported at least one adverse event, most of which were mild; no serious adverse events were noted in this study. No clinically significant changes in vital signs, physical examinations, electrocardiograms, or clinical biochemistry profiles were observed. The pharmacokinetic parameters of telavancin were similar between elderly men and women and comparable to historical results in healthy young subjects. No evidence was found to support telavancin dosage adjustment based on age or sex (Goldberg et al. 2010a).

Hepatic Impairment

In a phase I, open-label, single-dose, matched-control, pilot study, the pharmacokinetics of telavancin were compared between patients with moderate hepatic impairment and healthy controls. Eight adults with moderate hepatic impairment (Child–Pugh class B) and eight age-, sex-, and weight-matched healthy control subjects were studied. All participants received a single 1-h i.v. infusion of telavancin 10 mg/kg. Plasma samples were collected up to 72 h after the start of the infusion. Concentrations of telavancin and the most prevalent of several minor hydroxylated metabolites, THRX-651540, were assayed with a validated liquid chromatography-tandem mass spectrometry technique. Telavancin binding to plasma proteins was determined in a preinfusion sample by using equilibrium dialysis. Pharmacokinetic parameters for telavancin and THRX-651540 were generally similar between the hepatic impairment and control groups. The mean maximum plasma concentration was 21 % lower in patients with hepatic impairment than in controls, which was a statistically (analysis of variance, $P < 0.05$), but not clinically, significant difference. There were no other statistically significant between-group differences. Adverse events were few and mild. No apparent differences were observed in the pharmacokinetic disposition of telavancin in patients with hepatic impairment compared with healthy controls in this pilot study. The authors concluded that adjustment of the standard telavancin dosage regimen does not appear to be required in patients with mild-to-moderate hepatic impairment (Goldberg et al. 2010b).

Elimination

Elimination of Vancomycin

Virtually all of an i.v. administered dose of vancomycin is excreted by the kidneys in an unchanged form after 24 h. This occurs primarily by glomerular filtration, but there is evidence that some tubular secretion may occur as well (Moellering 1984; Rybak et al. 1990). About 80–90 % of an i.v. administered dose can be recovered from the urine during the first 24 h. Urine concentrations of 9–300 μg/mL are maintained for 24 h after a single 0.5-g i.v. dose in healthy adults (Geraci et al. 1956).

Geraci et al. (1956) found small quantities of vancomycin in the bile and feces after i.v. administration. After i.v. administration of vancomycin to children, Schaad et al. (1980) found fecal concentrations of the drug of 4.1–35.8 μg/g wet stool (mean, 12.5 μg/g). Extrarenal (possibly hepatic) excretion of vancomycin may occur to a small extent, because relatively high vancomycin clearances are observed in patients with compromised renal function (Rotschafer et al. 1982).

Because vancomycin administered i.v. is mainly eliminated by the kidneys, higher serum levels are produced in patients with impaired renal function than in those with normal function, if dosage modification is not employed. This may also apply to patients with liver disease.

Elimination of Teicoplanin

Renal clearance is the most important route of elimination. Most of the parenterally administered dose of teicoplanin is excreted in the urine as the active unchanged drug in 16 days. This accounts for about 83 % of the dose given. The total body clearance has been reported to be 11 mL/h/kg and is not dose dependent. Clearance of the unbound drug is by glomerular filtration and both tubular resorption and renal secretion are minimal.

Some 2.7 % of i.v. administered dose of active teicoplanin can be recovered from the feces (Buniva et al. 1988). This apparently is excreted into the bowel via the bile.

Administered teicoplanin, not excreted in urine or feces, is metabolized in the liver. Two metabolites, which arise due to hydroxylation of teicoplanin, have been identified in urine. They have some, but reduced activity against Gram-positive bacteria (Bernareggi et al. 1991, 1992).

In intravenous drug abusers, the renal (and non-renal) clearance of teicoplanin is increased (Rybak et al. 1991). In anuric patients undergoing CAPD, mean clearance rate was 10.68 mL/min, mean apparent volume of distribution was 0.80 L/kg, and mean volume of distribution at steady state was 0.22 L/kg. Mean dialysate excretion was 3.16 % and mean peritoneal clearance rate was 0.023 mL/min (Stamatiadis et al. 2003).

Elimination of Telavancin

Telavancin is primarily eliminated renally (70–80 %) with most of the drug being excreted unchanged in the urine (Wong et al. 2008). The remainder of the antibiotic undergoes minimal metabolism via hydroxylation.

Pharmacodynamics

Effects on the Pathogen

In Vitro Susceptibility of (Lipo)glycopeptides

The spectrum of activity of vancomycin, teicoplanin, and the newer lipoglycopeptides is similar. All show activity against Gram-positive pathogens such as staphylococci, streptococci, and enterococci and Gram-positive anaerobic bacteria including *Clostridium*, *Lactobacillus*, *Propionibacterium*, *Peptostreptococcus*, and *Corynebacterium* species (Goldstein et al. 2006). This activity is regardless of resistance to methicillin or penicillin. However, the major advantage in terms of the clinical spectrum of activity of these newer lipoglycopeptides over vancomycin is that telavancin is active against vancomycin-resistant organisms, including vancomycin-resistant enterococci and vancomycin-resistant *S. aureus* (VISA and VRSA) (Table 12.2) (Draghi et al. 2008a, b; Saravolatz et al. 2007; Krause et al. 2008; Mendes et al. 2012).

Telavancin activity against European *S. aureus* and coagulase negative staphylococci compared favorably with vancomycin, daptomycin, and linezolid (Mendes et al. 2011).

Leuthner et al. studied the activity of telavancin with and without the presence of serum against glycopeptide glycopeptide-intermediate staphylococcal species (GISS), heteroresistant GISS (hGISS), and three vancomycin-resistant *S. aureus* (VRSA) compared with vancomycin, quinupristin/dalfopristin, linezolid, and daptomycin. The MIC_{90} of 50 glycopeptide-intermediate *S. aureus* strains and heteroresistant vancomycin-intermediate *S. aureus* (hVISA) strains (vancomycin MIC_{90} 8 μg/mL) was 1 μg/mL or lower (Leuthner et al. 2006). Telavancin was also effective against all three strains of VRSA at clinically achievable concentrations of 4 μg/mL (Leuthner et al. 2006).

PK/PD Effects of Vancomycin

Reviews of pharmacokinetics and pharmacodynamics (PK/PD) have indicated the AUC/MIC as the pharmacodynamic index that best correlates with a successful outcome associated with the use of vancomycin based in part on data from animal models, in vitro studies, and limited human studies (Craig 2003; Rybak 2006).

Table 12.2 MIC_{90}s of glycopeptides and lipoglycopeptides against common Gram-positive organisms and range of MICs for VISA and VRSA

	Telavancin	Vancomycin	Linezolid	Daptomycin	Teicoplanin
Bacteria (no. of isolates)					
S. aureus, methicillin susceptible, MSSA ($n=1217$)[a], ($n=3764$)[b] ($n=1238$)[c]	0.5, 0.25, 0.25	1, –, 1	2, –, 2	0.5, –, 0.5	–, –, 1
S. aureus, methicillin resistant, MRSA ($n=1082$)[a], ($n=547$)[c], ($n=4278$)[b]	0.25, 0.5, 0.25	1, 2, 1	2, 2, 2	0.5, 1, 0.5	–, 4, ≥2
S. aureus, VISA ($n=6$)[d]	0.5–1	4–8	1–4	0.5–2	2–8
S. aureus, VRSA ($n=3$)[d]	2–4	>32	2	0.25–0.5	16–>32
Coag-negative staphylococci, methicillin susceptible ($n=100$)[a], ($n=60$)[c]	0.5, 0.25	2, 2	1, 1	1, 1	–, 4
Coag-negative staphylococci, methicillin resistant ($n=272$)[a], ($n=909$)[b] ($n=176$)[c],	0.5, 0.25, 0.5,	2, 2, 2	1, 1, 1	1, 0.5, 0.5	–, 8, 8
E. faecalis, vancomycin susceptible ($n=429$)[a], ($n=1376$)[b], ($n=426$)[c]	1, –, 0.5	2, –, 2	0.25, –, 2	1, –, 1	–, –, 0.25
E. faecalis, harboring VanA gene ($n=22$)[a], ($n=23$)[c]	16, 8	>512, >512	2, 1	1, 1	–, >128
E. faecium, vancomycin susceptible ($n=92$)[a], ($n=276$)[c]	0.25, 0.25	1, 1	2, 2	4, 4	–, 0.5
E. faecium, harboring VanA gene ($n=223$)[a], ($n=73$)[c]	8, 4	512, >512	2, 2	4, 4	–, >128
E. faecium, harboring VanB gene ($n=17$)[a]	2	0.5	1	4	–
S. pyogenes ($n=68$)[a], ($n=54$)[c]	0.06, 0.03	0.5, 0.5	1, 1	0.06, 0.06	–, –
S. agalactiae ($n=45$)[a], ($n=48$)[c]	0.06, 0.06	0.5, 0.5	1, 1	0.25, 0.25	–, –
Viridans group streptococci ($n=102$)[a], ($n=336$)[b] ($n=67$)[c]	0.12, 0.06, 0.12,	0.5, 1, 1	1, 1, 1	1, 1, 1	–, ≤2, –
S. pneumoniae, penicillin susceptible ($n=204$)[a], ($n=125$)[c]	0.03, 0.03	0.5, 0.5	1, 1	–, –	–, –
S. pneumoniae, penicillin nonsusceptible ($n=72$)[a], ($n=414$)[b], ($n=54$)[c]	0.015, –, 0.03	0.5, –, 0.5	1, –, 1	–, –, –	–, –, –

[a]Adapted from Draghi et al. (2008a) US isolates
[b]Adapted from Mendes et al. (2012)
[c]Adapted from Draghi et al. (2008b) isolates from Europe and Israel
[d]Adapted from Farrell et al. (2011), Mendes et al. (2012), Draghi et al. (2008a, b)

In Vitro

In in vitro studies, vancomycin has shown a concentration-independent effect. The bactericidal activities of vancomycin against two reference strains and two clinical isolates of *S. aureus* and *S. epidermidis* were studied with five different concentrations ranging from 2 to 64 times the MIC. The decrease in the numbers of CFU at 24 h was at least 3 $\log_{10}$ CFU/mL for all strains. No concentration-dependent killing was observed. The PAE was determined and was 1.2 h for *S. aureus* and 6.0 h for *S. epidermidis* (Lowdin et al. 1998). Mono- and biexponential killing curves for vancomycin over a 2–50 μg/mL concentration range were generated for 11 *S. aureus* isolates and 12 coagulase-negative staphylococcal spp. in the logarithmic phase of growth. Nonlinear least-square regression of the initial growth rate and disappearance were not significantly different for lower or higher concentrations of vancomycin in broth (Ackerman et al. 1992).

An in vitro pharmacodynamic system was used to demonstrate the concentration-independent pharmacodynamics of vancomycin against *S. aureus* ATCC 29213. Initial vancomycin concentrations of 5, 10, 20, and 40 μg/mL were studied monoexponentially while simulating a 6-h half-life. Time–kill curve analyses suggested that varying the concentration of vancomycin does not affect the rate or extent of bacterial killing aerobically or anaerobically against *S. aureus* and more efficient killing was achieved under aerobic conditions. The simulated distribution phase concentrations did not contribute to more effective killing of *S. aureus* ATCC 29213 (Larsson et al. 1996). In a study comparing vancomycin with daptomycin, two clinical MRSA and four hVISA were tested in an in vitro pharmacokinetic/pharmacodynamic model with simulated endocardial vegetations. Vancomycin regimens of 1 g every 12 h and 2 g every 12 h were utilized in a PK/PD model over 72 h. Vancomycin displayed minimal activity against MRSA isolates and minimal-to-no activity against hVISA. In general, the use of high-dose vancomycin over standard-dose vancomycin did not improve activity except against one of six isolates (MRSA 494) (Leonard and Rybak 2009). GISA strains have an increased inoculum effect in comparison with fully vancomycin-susceptible strains (LaPlante and Rybak 2004). MRSA bloodstream isolates from patients who had received vancomycin within the preceding 30 days had a significantly decreased vancomycin killing at 24 h in vitro (median $\log_{10}$ decrease, 3.1 vs. 2.2 CFU/mL; $P = 0.021$) and a significantly higher vancomycin MIC than isolates obtained from patients without that history ($P = 0.002$) (Moise et al. 2008). Vancomycin tolerance, defined as an MBC/MIC ratio ≥32 or an MBC/MIC ratio >16 associated with a resistant-level vancomycin MBC of >32 μg/mL, represents a lack of bactericidal activity (Geraci and Wilson 1981). A significant subset of *S. aureus* strains is associated with the risk of clinical failure due to vancomycin tolerance, regardless of the reported susceptibility levels (MICs). In a recent study of 213 *S. aureus* strains, 15 % of wild-type MRSA strains, 74 % of hVISA strains, and 100 % of VISA and VRSA strains were tolerant to vancomycin (Jones 2006). Tolerance (MBC/MIC ≥ 32) to vancomycin may also occur with strains of *S. epidermidis* (Geraci and Wilson 1981), viridans streptococci (Geraci and Wilson 1981; Meylan et al. 1986), *S. bovis* (Geraci and Wilson 1981), and

Group G streptococci (Noble et al. 1980). A pharmacokinetic–pharmacodynamic (PKPD) model that characterizes the full-time course of in vitro time–kill curve experiments of vancomycin was evaluated in its capacity to predict the previously determined PK/PD indices. A dose fractionation study was simulated, using a constant drug exposure of *S. pyogenes*. A bactericidal effect was not reached during the first 24 h of drug exposure and a *f*AUC/MIC of 54 was predicted to be needed for a bacteriostatic effect.

Patient Studies

There are very few human studies evaluating the pharmacodynamics of vancomycin, and the findings of most of those studies have not been conclusive in determining which parameter has the most value in predicting patient outcome. The majority of studies have involved relatively small patient populations and patients with a variety of infection types. In a retrospective analysis of vancomycin-treated patients with a *S. aureus*-associated lower respiratory tract infection, an AUC/MIC of >350 was associated with higher rates of clinical and bacteriological success than lower AUC/MIC values (Moise et al. 2000). In a subsequent publication, Moise-Broder et al. (2004a) showed that vancomycin AUC_{24}/MIC values predicted time-related clinical and bacteriological outcomes for patients with lower respiratory tract infections caused by methicillin-resistant *S. aureus*. Clinical and pharmacokinetic information on 108 patients (age range 32–93 years) with a *S. aureus* lower respiratory tract infection was used. Measured vancomycin AUC_{24}/MIC values were predicted with the software program AUIC calculator in a subset of these patients ($r^2 = 0.935$). Clinical and bacteriological response to vancomycin therapy was superior in patients with higher (≥400) AUC_{24}/MIC values ($P = 0.0046$). No relationship was identified between vancomycin $\%T > \text{MIC}$ and clinical response. Bacterial eradication of *S. aureus* (both methicillin-susceptible and methicillin-resistant) occurred more rapidly ($P = 0.04$) with vancomycin when a threshold AUC_{24}/MIC value was reached. *S. aureus* killing rates were slower with vancomycin than with other antistaphylococcal antibacterials ($P = 0.002$) (Moise-Broder et al. 2004a). A *f*AUC/MIC of 87.5–280 (AUC/MIC > 125–400) is associated with improved patient outcome with vancomycin therapy. However, an AUC/MIC target of >400 is very difficult to achieve without having what some consider "toxic" trough levels (Bosso et al. 2011).

A retrospective, single-center, observational cohort study was performed to determine whether vancomycin pharmacokinetic parameters as such (e.g., serum trough concentrations or AUC values) were associated with mortality for patients with healthcare-associated pneumonia (HCAP) attributed to MRSA. Patients with MRSA HCAP ($n = 102$) were identified over a 6.5-year period; 32 patients (31.4 %) died during their hospitalization. The mean (±s.d.) vancomycin trough concentrations (13.6 ± 5.9 vs. 13.9 ± 6.7 μg/mL, respectively; $P = 0.866$) and AUC values (351 ± 143 vs. 354 ± 10^9 μg h/mL, respectively; $P = 0.941$) did not differ between survivors and nonsurvivors. The stratification of the vancomycin trough concentrations and AUC values yielded no relationship with hospital mortality. The authors concluded that there was no evidence that greater vancomycin trough

concentrations or AUC values correlated with hospital outcome. However, MICs were not measured and therefore the AUC/MIC values are not known (Jeffres et al. 2006) and the power of this study was probably too small to draw any conclusions.

PK/PD Effects of Teicoplanin

The PK/PD parameter that is the most important and best predictor for the effects of treatment with teicoplanin is the AUC_{24h}/MIC, as it is for vancomycin (Craig 2003).

In Vitro Studies

In in vitro studies, concentration-dependent killing was noted against *S. epidermidis*, with a >4 log10 difference in CFUs between 2×MIC and 64×MIC at 24 h. Also, against *S. aureus* there was slight concentration-dependent killing, which, however, did not reach 2 log10 CFU/mL. Teicoplanin at 8×MIC exerted a similar killing rate at inocula of 5×10^3, 5×10^5, and 5×10^7 CFU/mL for *S. epidermidis*, except for slower initial killing up to 6 h at the highest inoculum. In contrast, overall slower killing at all inocula was seen for *S. aureus*, where an inoculum effect was noted at 5×10^7 CFU/mL. For *E. faecium*, only a bacteriostatic effect was noted at all concentrations and inocula (Odenholt et al. 2003).

Tolerance to vancomycin and teicoplanin in 90 clinical isolates of coagulase-negative staphylococci (CoNS) was investigated by time–kill curve methodology. Only six strains, belonging to the *S. lugdunensis* species, exhibited tolerance. The seven other *S. lugdunensis* strains tested displayed weak susceptibility to the bactericidal activity of glycopeptides compared to the other CoNS. These phenomena are of concern, since *S. lugdunensis* is recognized as one of the most pathogenic CoNS (Bourgeois et al. 2007).

Studies of the postantibiotic effect (PAE) and the postantibiotic sub-MIC effect (PASME) of teicoplanin showed no or very short PAEs for *S. epidermidis*, *S. aureus,* and *E. faecium*. However, when the strains in the postantibiotic phase were exposed to subinhibitory teicoplanin concentrations (0.1, 0.2, and 0.3×MIC) of teicoplanin (PASME), substantial prolongation of the PAEs was seen (Odenholt et al. 2003).

In an in vitro kinetic model following exposure to simulated human serum pharmacokinetic concentrations of teicoplanin (6 mg/kg OD at steady state) no significant killing was achieved for *S. epidermidis*, *S. aureus*, and *E. faecium*. Regrowth of *S. epidermidis* was noted first after 8 h, despite a $T>MIC_{24}$ of only 5 % (1.2 h), illustrating the long post-MIC effect for this strain. For *S. aureus*, $T>MIC$ was 38 %, and regrowth occurred later than for *S. epidermidis*. Neither killing nor regrowth was seen for *E. faecium* with a $T>MIC_{24h}$ of 27 % (Odenholt et al. 2003). In one in vitro study it was noted that teicoplanin alone (8 μg/mL) was usually bactericidal to teicoplanin-susceptible, but vancomycin-resistant *E. faecium* strains at 24 h, but only if these strains lacked high-level gentamicin resistance. If the latter was present, teicoplanin was inhibitory, but not bactericidal (Hayden et al. 1994).

The bacterial killing activity of teicoplanin against biofilms formed by two strains of *S. aureus* and two strains of *S. epidermidis* was assessed after exposure to antibiotics (1, 5, and 10 μg/mL) for 1, 3, 5, 7, 10, or 14 days using an in vitro model of biofilms on polyurethane film. The biofilms were completely sterile after exposure to teicoplanin (5 and 10 μg/mL) for 7 days. However, ciprofloxacin and rifampicin (both 5 μg/mL) achieved eradication of the biofilms of both staphylococcal species more rapidly than vancomycin or teicoplanin (Lee et al. 2006).

Animal Models

The PK/PD parameters of teicoplanin were also studied in animal models. In a mouse peritonitis model of immunocompetent mice infected with *S. aureus* and *S. pneumoniae*, a wide spectrum of different treatment regimens was tested. In studies in which the single dose that protected 50 % of lethally infected mice (ED_{50}) was given as one dose or was divided into two doses, survival was significantly decreased when the dose was divided. The only statistically significant correlations between the percentage of survival of the mice after 6 days and each of the PK/PD parameters were for peak concentration (C_{max})/MIC and *S. aureus*, and for the free fraction of C_{max} ($C_{max\text{-}free}$)/MIC and *S. pneumoniae*. For *S. pneumoniae*, the ED_{50} for different dosing regimens increased with the number of doses given, e.g., the single-dose ED_{50} for teicoplanin was 0.45 mg/kg, but the ED_{50} for dosing regimens with 2-h doses given for 48 h was 5.67 mg/kg. In experiments with 40 different teicoplanin dosing regimens against *S. pneumoniae*, the different PK/PD parameters were analyzed using logistic regression. The $C_{max\text{-}free}$/MIC was one of two parameters that best explained the effect, the other parameter was the time the free fraction of the drug is above the MIC. The effect analyzed as a function of $C_{max\text{-}free}$/MIC disclosed thresholds with shifts from almost no effect to full effect at ratios of five 2–3 for teicoplanin (Knudsen et al. 2000).

Patient Studies

In a prospective, randomized, crossover study in the surgical ICU, i.v. administration of teicoplanin was compared to s.c. administration. Patients received a loading dose of 6 mg/kg per 12 h for 48 h i.v. and then continued at a daily dose of 6 mg/kg. Compared with a 30-min i.v. infusion the peak concentration of teicoplanin after a 30-min s.c. administration occurred later (median 7 h, range 5–18) and was lower (16 μg/mL, 9–31; vs. 73 μg/mL, 53–106). Despite large and unpredictable interindividual differences, no significant differences between s.c. and i.v. administration were observed in trough antibiotic concentrations (10 μg/mL, 6–24; vs. 9 μg/mL, 5–30), the $AUC_{0\text{–}24\ h}$ (309 μg/mL/min, 180–640; vs. 369 μg/mL/min, 171–955), the proportion of the dosing interval during which the plasma teicoplanin concentration exceeded 10 μg/mL (96 %, 0–100 %; vs. 79 %, 13–100 %), and the ratio of $AUC_{0\text{–}24\ h}$ to 10 (77, 45–160; vs. 92, 43–239) (Barbot et al. 2003).

Logistic regression analysis was performed on data drawn from a clinical trials database for *S. aureus* septicemia treated with teicoplanin. Variables analyzed were age, body weight, mean predose and postdose serum teicoplanin concentrations, mean dose (mg or mg/kg body weight), and combination versus monotherapy. Only two variables correlated significantly with clinical outcome: age ($P=0.012$) and mean predose serum concentration ($P=0.010$). The probability of successful treatment declined with age and increased with mean predose serum concentration (Harding et al. 2000).

In an observational study, C_{min} teicoplanin levels were determined for the first 4 days of treatment following administration of loading doses of 6 mg/kg every 12 h on day 1 followed by 6 mg/kg once or twice daily. The two target trough values (≥10 μg/mL and ≥20 μg/mL) were only achieved by day 4 in the once-daily group ($n=34$; mean 9.55 μg/mL, 95 % CI 8.17–10.94 μg/mL) and in the twice-daily group ($n=40$; mean 21.8 μg/mL, 95 % CI 17.21–26.39 μg/mL), respectively. The mean C_{min} in the twice-daily group was ≥10 μg/mL (11.03 μg/mL) by day 2. Teicoplanin loading doses of 6 mg/kg every 12 h for 48 h followed by 6 mg/kg once-daily would be sufficient for infections other than infective endocarditis, septic arthritis, and osteomyelitis (Brink et al. 2008). Teicoplanin levels of ≥20 μg/mL for bone and joint infection in stable adult patients are best achieved with a daily dose of at least 600 mg. A trough serum teicoplanin level of ≥20 μg/mL was predictive of improved outcomes in observational studies of serious staphylococcal infection (Greenberg and Benes 1990). Prospectively collected data for 141 clinically stable adults with bone and joint infection treated as outpatients with teicoplanin 400 mg or 600 mg i.v. once daily showed that 51 % of trough levels, which were available for 78 % of episodes, were ≥20 μg/mL. There was no significant relationship between teicoplanin level and age, body weight, or creatinine clearance, but male gender was associated with lower trough levels than female gender ($P=0.03$) (Matthews et al. 2007).

Pharmacodynamic exposures, measured as the ratio of steady-state total drug area under the curve to MIC (AUC/MIC), were modeled using a 5,000-patient Monte Carlo simulation against 119 nonduplicate clinical isolates of *S. aureus* and 82 coagulase-negative staphylococci (CNS) collected from hospitals in Brazil between 2003 and 2005. Pharmacodynamic targets included an AUC/MIC > 345 for teicoplanin. The cumulative fractions of response (CFRs) against all *S. aureus* isolates were 30.1 % and 71.6 % for teicoplanin 400 mg every 24 h and 800 mg every 24 h, respectively. CFRs against all CNS isolates were 13.4 % and 34.6 %, respectively. The CFR was reduced among the methicillin-resistant isolates. Higher doses of teicoplanin increased the CFR (Kuti et al. 2008).

PK/PD Effects of Telavancin

Telavancin is a concentration-dependent drug with rapid bactericidal activity against Gram-positive bacteria. The AUC/MIC is suggested as the best predictor of efficacy for telavancin (Hegde et al. 2004).

In Vitro

In a time–kill study, concentration-dependent activity was displayed at different concentrations above the MICs and displayed maximum decreases in colony-forming units at 24 h for all concentrations tested. When tested against a variety of *S. aureus* isolates, including heteroresistant vancomycin-intermediate *S. aureus* (hVISA), VISA, and VRSA, telavancin maintained concentration-dependent activity (Leuthneret al. 2006). In another in vitro study, both C_{max}/MIC and AUC/MIC (total drug) were correlated with telavancin antimicrobial activity (Lubenko et al. 2008).

The in vitro postantibiotic effect (PAE) of telavancin is 4–6 h (Pace et al. 2003), which is approximately four times longer than that of vancomycin against staphylococci.

Extended in vitro postantibiotic effects of telavancin were demonstrated by Pankuch and Appelbaum. The in vitro postantibiotic effects (PAEs), postantibiotic sub-MIC effects (PA-SMEs), and sub-MIC effects of telavancin were determined for 16 Gram-positive organisms. Telavancin staphylococcal, streptococcal, and enterococcal PAE ranges were 0.9–3.9 h, 0.4–6.7 h, and 0.3–2.2 h, respectively. The PA-SME ranges (0.4 times the MIC) for staphylococci, streptococci, and enterococci were 6.7 to >10.7 h, >10.7 to >11.0 h, and >10 to >10.8 h, respectively (Pankuch and Appelbaum 2009).

The serum bactericidal activity of telavancin was tested during the phase I pharmacokinetic studies (Shaw et al. 2005). In support of once-daily dosing of telavancin, serum bactericidal titers (SBTs) were ≥16 at 24 h after dosing, but greater SBTs against both MRSA and *Streptococcus pneumoniae* strains were achieved with higher doses of telavancin.

The intracellular bactericidal activity of telavancin was −3 log (MSSA) to −1.5 log (VRSA) at C_{max} and at 24 h, significantly lower than the extracellular activity. A bimodal relationship with respect to concentration (at 24 h) was observed for both MSSA and MRSA. In contrast, vancomycin exhibited only marginal intracellular activity towards intraphagocytic MSSA, MRSA, and VISA (max. −0.5 log decrease at 24 h and at C_{max}) (Barcia-Macay et al. 2006).

In an in vitro PK/PD model with simulated endocardial vegetations, telavancin demonstrated greater killing than vancomycin including a hGISA and a GISA strain (Leonard and Rybak 2009).

The antibacterial effects of telavancin, against six *S. aureus* strains (one MSSA strain, four MRSA strains, and one vancomycin-intermediate *S. aureus* [VISA] strain) and three *Enterococcus* sp. strains (one *E. faecalis* strain, one *E. faecium* strain, and one vancomycin-resistant *E. faecium* [VREF] strain) were compared to vancomycin, and teicoplanin using an in vitro pharmacokinetic model of infection. Analyzing the data from all five vancomycin-susceptible *S. aureus* (VSSA) strains or all four MRSA strains showed that telavancin was superior in its antibacterial effect as measured by the area under the bacterial kill curve at 24 h ($AUBKC_{24}$) and 48 h ($AUBKC_{48}$) in comparison to vancomycin or teicoplanin ($P < 0.05$). Telavancin was also superior to vancomycin and teicoplanin in terms of its greater early killing

effect ($P<0.05$). Against the three Enterococcus spp. tested, telavancin was superior to vancomycin in terms of its $AUBKC_{24}$, $AUBKC_{48}$, and greater early bactericidal effect ($P<0.05$). Dose-ranging studies were performed to provide free-drug area under the concentration–time curve over 24 h in the steady state divided by the MIC (*f*AUC/MIC) exposures from 0 to 1,617 (7–14 exposures per strain) for five VSSA, four VISA, and the three Enterococcus strains. The *f*AUC/MIC values for a 24-h bacteriostatic effect and a 1-log-unit drop in the viable count were 43.1 ± 38.4 and 50.0 ± 39.0 for VSSA, 3.2 ± 1.3 and 4.3 ± 1.3 for VISA, and 15.1 ± 8.8 and 40.1 ± 29.4 for the Enterococcus spp., respectively. The reason for the paradoxically low *f*AUC/MIC values for VISA strains is unknown. There was emergence of resistance to telavancin in the dose-ranging studies, as indicated by subpopulations able to grow on plates containing 2× MIC telavancin concentrations compared to the preexposure population analysis profiles. Changes in population analysis profiles were less likely with enterococci than with *S. aureus*, and the greatest risk of changed profiles occurred for both species at *f*AUC/MIC ratios of 1–10. Maintaining a *f*AUC/MIC ratio of >50 reduced the risk of subpopulations able to grow on antibiotic-containing media emerging. The authors concluded that these data help explain the clinical effectiveness of telavancin against MRSA and indicate that telavancin may have clinically useful activity against Enterococcus spp., and perhaps also VISA, at human doses of 10 mg/kg/day. In addition, they support a clinical breakpoint of susceptible at <1 μg/mL for both *S. aureus* and *Enterococcus* spp. (MacGowan et al. 2011).

Lubenko et al. compared the pharmacodynamics of telavancin and vancomycin with *S. aureus* in an in vitro dynamic model. Concentrations were simulated between the MIC and the mutant *prevention concentration (MPC), and above the MPC*. Two strains of *S. aureus*, glycopeptide-intermediate *S. aureus* (GISA) Mu-50 and ATCC 43300, were exposed for 5 days to once-daily telavancin and twice-daily vancomycin. The simulated ratios of AUC_{24} to MIC varied from 30–50 to 3,400 h. The cumulative antimicrobial effect was expressed by ABBC (area between the level corresponding to the starting inoculum and the time–kill curve calculated from time 0 to 144 h). With each antibiotic, the ABBC versus log AUC_{24}/MIC relationships were bacterial strain independent. A sigmoid model fits combined data on both organisms exposed to telavancin ($r^2=0.78$) or vancomycin ($r^2=0.85$). Comparable effects of the proposed therapeutic dose of telavancin (10 mg/kg) and a clinical dose of vancomycin (2 × 1 g) were predicted for MRSA ATCC 43300 (AUC_{24}/MIC 3,400 and 500 h, respectively) and a 1.6-fold greater effect of telavancin for GISA Mu-50 compared with vancomycin (AUC_{24}/MIC 1,700 and 130 h, respectively). Mutants of *S. aureus* ATCC 43300 resistant to 2× and 4× MIC of vancomycin but not telavancin were enriched in these simulations. No selection of telavancin- and vancomycin-resistant mutants of GISA Mu-50 was observed. The authors concluded that these data suggest that the effects of clinically attainable AUC/MIC ratios of telavancin are similar to those of vancomycin on *S. aureus* 43300 and twofold greater on GISA Mu-50 (Lubenko et al. 2008).

Animal Models

The in vivo pharmacodynamics of telavancin with Gram-positive organisms were evaluated in the mouse neutropenic thigh (MNT) and mouse subcutaneous infection (MSI) animal models. PK/PD studies in the MNT model demonstrated that the AUC_{24h}/MIC ratio was the best predictor of efficacy. Telavancin produced dose-dependent reduction of thigh titers of several organisms, including MSSA and MRSA, penicillin-susceptible and -resistant strains of *S. pneumoniae*, and vancomycin-resistant *E. faecalis*. The 50 % effective dose (ED_{50}) estimates for telavancin ranged from 0.5 to 6.6 mg/kg of body weight (administered i.v.), and titers were reduced by up to 3 log10 CFU/g from pretreatment values. Against MRSA ATCC 33591, telavancin was 4- and 30-fold more potent (on an ED_{50} basis) than vancomycin and linezolid, respectively. Against MSSA ATCC 13709, telavancin was 16- and 40-fold more potent than vancomycin and nafcillin, respectively. Telavancin, vancomycin, and linezolid were all efficacious and more potent against MRSA ATCC 33591 in the MSI model compared to the MNT model. This deviation in potency was, however, disproportionately greater for vancomycin and linezolid than for telavancin, suggesting that activity of telavancin is less affected by the immune status. These studies provided the evidence for once-daily dosing of telavancin (Hegde et al. 2004).

In two studies, the pharmacodynamics of telavancin were compared with vancomycin using a model of aortic valve endocarditis. Rabbits were infected with *S. aureus* (MRSA vs. VISA and two different strains of VISA) (Madrigal et al. 2005; Miro et al. 2007). One study described rapid sterilization of vegetations with 2 days of therapy at a human dose simulation of 7.5 mg/kg/day telavancin (Madrigal et al. 2005). Telavancin was as active as vancomycin against MRSA and more active than vancomycin against the VISA strain. Similar results were found in the other study with telavancin dose simulating 10 mg/kg i.v. every 24 h (Miro et al. 2007). Telavancin reduced vegetation titers and sterilized vegetations more effectively than vancomycin for two VISA strains; however, the difference was not statistically significant.

Telavancin was compared with vancomycin and linezolid in a neutropenic murine model of MRSA pneumonia (Reyes et al. 2005). Telavancin quickly achieved >3-log decrease in lung bacterial titer within 8 h against MRSA (MIC 0.5 μg/mL), but vancomycin (MRSA MIC of 1 μg/mL) required up to 24 h to produce the same effect. Unlike vancomycin and telavancin, linezolid (MRSA MIC 1 μg/mL) displayed bacteriostatic activity.

The efficacies of telavancin and vancomycin against MRSA strains with vancomycin MICs of ≥1 μg/mL were compared in a neutropenic murine lung infection model. Thirteen clinical MRSA isolates (seven vancomycin-susceptible, two vancomycin-heteroresistant (hVISA), and four vancomycin-intermediate (VISA) isolates) were tested after 24 h, and seven isolates (one hVISA and four VISA isolates) were tested after 48 h of exposure. Mice were administered s.c. doses of telavancin at 40 mg/kg of body weight every 12 h (q12h) or of vancomycin at 110 mg/kg q12h; doses were designed to simulate the area under the concentration–time curve for the free, unbound fraction of drug (*f*AUC) observed for humans given

telavancin at 10 mg/kg q24h or vancomycin at 1 g q12h. Efficacy was expressed as the 24- or 48-h change in lung bacterial density from pretreatment counts. At dose initiation, the mean bacterial load was 6.16 ± 0.26 log10 CFU/mL, which increased by averages of 1.26 ± 0.55 and 1.74 ± 0.68 log in untreated mice after 24 and 48 h, respectively. At both time points, similar CFU reductions were noted for telavancin and vancomycin against MRSA, with vancomycin MICs of ≤2 μg/mL. Both drugs were similarly efficacious after 24 and 48 h of treatment against the hVISA strains tested. Against VISA isolates, telavancin reduced bacterial burdens significantly more than vancomycin for one of four isolates after 24 h and for three of four isolates after 48 h. These data support the potential utility of telavancin for the treatment of MRSA pneumonia caused by pathogens with reduced susceptibility to vancomycin (Crandon and Nicolau 2011).

In a rabbit meningitis model, the standard regimen, ceftriaxone plus vancomycin, was compared with telavancin against penicillin-resistant *S. pneumoniae* (PRSP) (Stucki et al. 2006). Additionally, the efficacy for MSSA meningitis was evaluated using telavancin versus vancomycin. Penetration of unbound telavancin is approximately 2 % into inflamed meninges and negligible into noninflamed meninges. Although telavancin displayed a low CSF/MIC ratio, telavancin was effective in the sterilization of the CSF in 60 % of the rabbits in both groups. The investigators suggested that telavancin monotherapy is more effective in PRSP meningitis than the standard regimen (ceftriaxone plus vancomycin) and is as effective as vancomycin monotherapy against MSSA meningitis.

In a neutropenic murine model of bacteremic peritonitis, the telavancin group showed only a 7 % mortality over 2 weeks compared with the vancomycin group (100 %) and a control group (100 %). Additionally, telavancin reduced bacterial titers in the blood and spleen by a greater amount compared with vancomycin therapy (Reyes et al. 2006).

The PK/PD profiles of the Food and Drug Administration (FDA)-approved telavancin renal dose adjustment schemes were studied by Lodise et al. A previously published two compartment open model with first-order elimination and a combined additive and proportional residual error model derived from 749 adult subjects in 11 clinical trials was used to simulate the individual concentration–time profiles for 10,260 subjects (NONMEM). The dosing regimens simulated were: 10 mg/kg once-daily for individuals with $CL_{CR} > 50$ mL/min, 7.5 mg/kg once-daily for individuals with CLCR between 30 and 50 mL/min, and 10 mg/kg every 2 days for those with $CL_{CR} < 30$ mL/min. The AUC under one dosing interval (AUCτ) was computed as dose/CL. The probability of achieving an AUCτ/MIC ratio ≥219 was evaluated separately for each renal dosing scheme. Evaluation of the dosing regimens demonstrated similar AUC values across the different renal function groups. For all renal dosing strata, >90 % of the simulated subjects achieved an AUCτ/MIC ratio ≥219 for MIC values as high as 2 μg/mL. For patients with $CL_{CR} < 30$ mL/min, the probability of target attainment (PTA) exceeded 90 % for both the $AUC_{0–24\,h}$ and $AUC_{24\text{-}48h}$ intervals for MICs ≤ 1 μg/mL. At a MIC of 2 μg/mL, the PTA was 89.3 % and 23.6 % for the $AUC_{0–24\,h}$ and $AUC_{24\text{-}48h}$ intervals, respectively. The comparable

PTA profiles for the three dosing regimens across their respective dosing intervals indicates that the dose adjustments employed in phase III complicated skin and skin structure infection trials were appropriate (Lodise et al. 2012).

Effects on the Host: Concentration-Related Toxicity

Toxicity of Vancomycin

Although long considered to be toxic to kidneys, the true potential of vancomycin to cause kidney damage is not entirely clear. Early reports of nephrotoxicity may have been related to impurities. Other factors that affect renal function, such as other nephrotoxic drugs (particularly aminoglycosides), hypotension, and a variety of underlying medical conditions are often present in patients being treated with vancomycin, and these factors confound the interpretation of most studies examining the nephrotoxicity of vancomycin.

In patients receiving vancomycin alone, varying rates of nephrotoxicity have been reported. Nephrotoxicity was not observed in any of 25 patients reported by Sorrell and Collignon (1985), while Farber and Moellering (1983), in a retrospective study, found that only 5 % of patients receiving vancomycin alone developed renal impairment, and this was reversible on discontinuation of the drug. A similar figure was obtained by Rybak et al. (1990) in a prospective study of 168 adult patients with no underlying or predisposing factors that might affect renal function. Higher rates of nephrotoxicity have been noted by other authors: 13 and 19 % in two studies of elderly patients (Downs et al. 1989; Goetz and Sayers 1993) and 15 % in cancer patients (Cimino et al. 1987). Dean et al. (1985) detected rises in serum creatinine in two of 19 children treated with vancomycin. Vancomycin-related nephrotoxicity in neonates is rare, and no clear relation to serum concentrations has been demonstrated (de Hoog et al. 2004).

The data on the nephrotoxicity of vancomycin combined with an aminoglycoside are also conflicting. Farber and Moellering (1983) noted a renal impact in 35 % of patients treated with vancomycin and an aminoglycoside. Pauly et al. (1990) found that 28 of 105 patients (27 %) treated with combined vancomycin and an aminoglycoside developed renal impairment, although another nephrotoxic factor was present in 22 of these 28 patients. Rybak et al. (1990) reported renal impairment in 14 of 63 patients (22 %) treated with vancomycin and an aminoglycoside, compared with only 8 of 168 patients (5 %) receiving vancomycin alone (see above). Other studies do not support a significant nephrotoxic interaction between vancomycin and an aminoglycoside. Neither Mellor et al. (1985) nor Cimino et al. (1987) found evidence of synergistic nephrotoxicity between the two drugs, and Goetz and Sayers (1993) noted only a mild increase in nephrotoxicity with vancomycin and an aminoglycoside (24 %) versus vancomycin alone (19 %). In a detailed prospective study of 289 patients, Vance-Bryan et al. (1993, 1994) reported an overall rate of vancomycin nephrotoxicity of 13.4 %. Nephrotoxicity was significantly more common in patients older than 60 years (18.9 %) compared with patients aged less than 60 years (7.8 %). Associations were noted with loop diuretic use in the elderly and

with amphotericin B use in the younger population. However, aminoglycoside use did not significantly increase the risk of nephrotoxicity in either age group or in the overall population. Goetz and Sayers (1993) performed a meta-analysis of nine other studies published between 1966 and 1991 of adult patients receiving vancomycin plus an aminoglycoside or either agent alone. Of patients receiving vancomycin alone, 8.1 % developed renal impairment, compared with 21.4 % of those treated with vancomycin and an aminoglycoside. Although this difference was highly significant, 17.1 % of patients in five of these studies treated with an aminoglycoside alone developed renal impairment. The authors point out an obvious problem in the interpretation of these nonrandomized studies, namely that receipt of combination therapy may be a marker for more serious underlying conditions, and that these conditions, rather than vancomycin, are responsible for nephrotoxicity. The only data on the nephrotoxicity of vancomycin in combination with an aminoglycoside in randomly allocated patients come from randomized studies of neutropenic patients (EORTC 1991). Of 370 patients treated with ceftazidime and amikacin, only two developed renal impairment, compared with 6 of 383 treated with vancomycin plus these two drugs.

Some early studies have associated high trough levels with the development of nephrotoxicity (Farber and Moellering 1983; Rybak et al. 1990; Cimino et al. 1987), but nephrotoxicity has also occurred in patients who have trough serum levels within the normal range. Moreover, the increased vancomycin levels that may be seen in association with renal impairment have not always preceded an increase in serum creatinine and so may be secondary to the renal impairment and not its primary cause (Cantu et al. 1994). The concentration-related nephrotoxicity of vancomycin has been studied in a number of hospitals (Hidayat et al. 2006; Jeffres et al. 2006; Lodise et al. 2008b; Kullar et al. 2011; Pritchard et al. 2010). Most were retrospectively performed. They have focused on either trough concentrations or the magnitude of total daily dose, and most determined that it is the trough concentration that best describes the drug exposure–toxicity relationship (Lodise et al. 2009). Higher rates of nephrotoxicity (11–35 %) were observed in these studies. Recently, the relative incidence of nephrotoxicity in relation to trough concentration was prospectively assessed in patients with documented MRSA infections in a multicentre setting. At seven US hospitals, adult patients ($n=288$) receiving vancomycin for at least 72 h with at least one vancomycin trough concentration determined under steady-state conditions were studied. The relationship between vancomycin trough concentrations of >15 μg/mL and the occurrence of nephrotoxicity was assessed using univariate and multivariate analyses, controlling for age, gender, race, dose, length of therapy, use of other nephrotoxins (including contrast media), intensive care unit (ICU) residence, episodes of hypotension, and comorbidities. Nephrotoxicity was defined as an increase in serum creatinine of 0.5 mg/dL or a >50 % increase from the baseline for two consecutive measurements. Nephrotoxicity was observed for 42 patients (29.6 %) with trough concentrations >15 μg/mL and for 13 (8.9 %) with trough concentrations of <15 μg/mL. Multivariate analysis revealed vancomycin trough concentrations of >15 μg/mL and race (black) as independent risk factors for nephrotoxicity in this population. No data on reversibility were presented in this report (Bosso et al. 2011).

Ingram et al. (2008) analyzed risk factors for nephrotoxicity in 102 patients receiving continuous vancomycin infusion as outpatient parenteral antibiotic therapy (OPAT). The likelihood of developing nephrotoxicity (≥50 % increase in serum creatinine from baseline) was evaluated in relation to demographic variables, underlying comorbidities, infectious disease diagnoses, concomitant drug exposures, and vancomycin concentration. The majority of the patients (66.7 %) were treated for bone and joint infection. The cumulative incidence of nephrotoxicity was 15.7 %. Nephrotoxicity was found to be associated with hypertension (OR 5.302 (95 % CI, 1.159–24.246), $P=0.031$), exposure to aminoglycosides (OR 6.594 (95 % CI, 1.026–42.385), $P=0.047$), loop diuretics (OR 8.123 (95 % CI, 1.449–45.528), $P=0.017$), and steady-state vancomycin concentration ≥28 μg/mL (OR 21.236 (95 % CI, 2.687–167.857), $P=0.004$) (Ingram et al. 2008).

Early reports described tinnitus and deafness complicating vancomycin treatment. However, many of these patients were being treated with other ototoxic drugs, such as streptomycin or erythromycin. It is also possible that earlier preparations of vancomycin were more toxic to the ear, just as these preparations may have been responsible for more hypersensitivity reactions and renal damage. Although many case reports and small series of cases of deafness related to vancomycin have subsequently appeared in the literature, ototoxicity does not appear to be common. It was not observed in a retrospective study of 98 vancomycin-treated patients (Farber and Moellering 1983) and of 54 patients monitored prospectively by Sorrell and Collignon (Sorrell and Collignon 1985), only 1 of 11 tested patients, who was also being treated with gentamicin, developed unilateral hearing impairment. Mellor et al. (1985) followed 34 patients prospectively and temporary tinnitus and deafness developed in two patients, one of whom also received gentamicin. A review of the literature (Brummett and Fox 1989) concluded that the ototoxicity of vancomycin has been overrated, and that only a very few cases of true vancomycin ototoxicity have occurred. However, an animal study has demonstrated that vancomycin, though not ototoxic itself (Lutzet al. 1991), enhances the ototoxicity of aminoglycosides (Brummett et al. 1990), so vancomycin may be ototoxic when used with aminoglycosides, or possibly with other ototoxic agents.

A relationship between serum levels of vancomycin and ototoxicity has not been established. High peak levels of 80 μg/mL were reported to be associated with auditory toxicity in patients with renal failure (Lindholm and Murray 1966), but high frequency hearing loss and tinnitus have also occurred with peak serum levels as low as 38–40 μg/mL (Sorrell and Collignon 1985). In a retrospective case–control study, audiometry results of 89 patients with available baseline and follow-up examinations on vancomycin therapy were analyzed. After an average of 27 days of vancomycin therapy, 89 patients showed a 12 % rate of high frequency hearing loss, with a trend towards a higher rate with advanced age. The mean of the highest vancomycin trough concentration for both patients with worsening and those without worsening audiograms was 19 μg/mL. Regression tree modeling identified that of patients aged <53 years, the incidence was 0 %, while for patients ≥53 years of age, the rate of high frequency hearing loss detected on audiogram was 19 % ($P=0.008$) (Forouzesh et al. 2009).

Toxicity of Teicoplanin

In animals teicoplanin, similar to vancomycin, causes a dose-related nephrotoxicity (Marre et al. 1987). In humans nephrotoxicity appears to be less common with teicoplanin than with vancomycin and this also applies when either of the two drugs are administered together with an aminoglycoside (Smith et al. 1989; Davey and Williams 1991; Van der Auwera et al. 1991). In one clinical trial, coadministration of vancomycin plus cyclosporin A was more nephrotoxic than teicoplanin plus cyclosporin A, but among patients receiving vancomycin plus amphotericin B and those treated by teicoplanin plus amphotericin B, deterioration in renal function was equal in both groups (Kureishi et al. 1991). One study in 100 consecutive neutropenic patients with hematological malignancies and persistent fever compared the toxicity of teicoplanin and vancomycin. A multivariate analysis revealed that concomitant use of amphotericin B ($P<0.001$) and treatment with vancomycin ($P=0.002$) were independently associated with nephrotoxicity. If combined with amphotericin B—vancomycin was significantly more nephrotoxic than teicoplanin (Hahn-Ast et al. 2008).

The potential for ototoxicity of teicoplanin has been studied. Studies in guinea pigs revealed no ototoxicity either with teicoplanin alone or combined with ethacrynic acid, a diuretic which augments the ototoxicity of many drugs (Brummett et al. 1987). In patients, ototoxicity with teicoplanin appears to be rare. A few patients receiving high-dose (15 mg/kg) teicoplanin daily developed tinnitus or a mild loss of high-frequency hearing detected by audiograms. The peak teicoplanin serum levels in these patients were 85 μg/mL and trough levels 41 μg/mL (Greenberg 1990). Of 3,377 patients treated with teicoplanin, 11 developed some degree of ototoxicity but other factors, rather than teicoplanin, might have caused this side effect in some of these patients (Davey and Williams 1991). Audiometry over time was performed on 17 patients who were treated either with teicoplanin or cloxacillin for severe staphylococcal infections. The hearing thresholds of 12 patients treated with teicoplanin showed a slight but significant increase over time. The authors stated that previous reports potentially underestimated the risk (Bonnet et al. 2004).

Toxicity of Telavancin

The following adverse events have been reported throughout clinical trials with telavancin: taste disturbances, headaches, QTc interval changes, insomnia, dizziness, nausea, mild rash, infusion-associated reactions, and serum creatinine elevation.

A phase I clinical study assessed the safety of telavancin 0.25–15 mg/kg in 54 healthy individuals (Shaw et al. 2005). The most commonly reported adverse events were mild taste disturbance (75 % in telavancin group vs. 14 % in placebo group) and headache (40 % vs. 29 %); other reported adverse events included dizziness (35 %), nausea (20 %), infusion-associated reactions (two subjects), and mild rash (two subjects). Although the study was not designed to detect QTc prolongation, reports of changes in the QTc interval observed at dosages ≥10 mg/kg were of

greater magnitude in the telavancin group than in those receiving placebo. In a separate phase I study, QTc interval changes were evaluated at two dosages (7.5 and 15 mg/kg) of telavancin vs. placebo or moxifloxacin for 3 days in 160 healthy individuals (Barriere et al. 2004). The moxifloxacin group had more than two times higher QTc prolongation than the telavancin groups. Within the telavancin groups, there was no difference in QTc change. Overall, no subject had a QTc > 500 ms or experienced a cardiovascular adverse event. During phase II trials, there was a similar incidence of adverse events between the telavancin and the standard treatment groups (6 % vs. 5 %) (Stryjewski et al. 2005). However, there were more cases of reversible, elevated serum creatinine (seven versus two patients) and mild, transient platelet count decrease (seven versus no patients) among patients receiving telavancin. Subjects with decreased platelet counts did not experience clinical significant bleeding events. During the FAST-2 study on cSSSI, only 6 % and 3 % receiving telavancin and standard therapy, respectively, were discontinued because of events. However, 73 % and 59 % of reported adverse events were defined to be possibly or probably related to telavancin and the standard therapy, respectively (Stryjewski et al. 2006). A few subjects ($n = 5$) in the telavancin group experienced reversible elevated serum creatinine (≤1.8 mg/dL) and reversible hypokalemia. The mean QTc change from baseline was 12.5 ms longer in the telavancin group than in the standard therapy group ($P \leq 0.0001$), no cardiovascular effects were seen.

In the phase III cSSSI study ATLAS I ($n = 855$ patients), no differences in serious adverse events were noted between the telavancin and vancomycin groups (Corey et al. 2009). The safety results of two pneumonia phase III studies (ATTAIN I and II, $n = 1{,}506$ patients), showed similar rates of adverse events in the telavancin and vancomycin groups (Rubinstein et al. 2011) The most common adverse events with telavancin were similar to other studies. Increases in serum creatinine level were more common with telavancin (16 % vs. vancomycin 10 %). QTc changes were similar in the two treatment groups. Both groups included a proportion of patients with ≥60 ms postbaseline changes in QTc or QTc results maximizing ≥500 ms.

Of note, due to a higher risk of mortality in patients with $CL_{CR} < 30$ mL/min, EMA's Committee for Medicinal Products for Human Use (CHMP) considers the use in telavancin contraindicated in patients with a $CL_{CR} < 30$ mL/min (Telavancin EMA product information 2011).

Implementation of PK/PD Knowledge into Clinical Practice

Vancomycin: Higher Doses and Continuous Infusion

Since failures have been described during treatment of MRSA infections with vancomycin, various new dosing approaches have been considered to improve clinical efficacy. These include the administration of increased doses to achieve higher serum trough concentrations and the administration of vancomycin by continuous

infusion. To study the correlation between elevated vancomycin MIC and treatment failure, the findings of a large multicenter phase III and IV prospective study were analyzed. Vancomycin failure rates of 22, 27, and 51 % were observed for patients infected with MRSA strains that had MICs of 0.5, 1.0, and 2.0 μg/mL, respectively (Moise-Broder et al. 2004a, b). In a related study, analyzing data on a subset of bacteremic patients to whom vancomycin was given in doses to achieve a trough concentration of 10–15 μg/mL, the authors demonstrated reduced vancomycin bactericidal activity in vitro for susceptible strains with a higher vancomycin MIC (Sakoulas et al. 2004). Similarly, Charles et al. (2004) noted a statistical correlation between the presence of hVISA—as detected by population analysis profile (PAP) testing—bacteremia and preceding lower serum vancomycin serum levels in infected patients. Soriano et al. (2008) evaluated vancomycin efficacy in MRSA bacteremia by MIC and showed a significantly higher mortality when vancomycin was used empirically and the vancomycin MIC was 2 μg/mL—an MIC at the upper end of the susceptible range (Soriano et al. 2008). However, the routinely derived MIC for an isolate may not be the most accurate means of identifying reduced vancomycin susceptibility and alternative laboratory methods, such as PAP testing or use of heavy inoculum E-test, may be more accurate (Hiramatsu 2001; Howden et al. 2004; Wootton et al. 2007). A retrospective quasi experimental study showed that consensus guidelines' suggested targets of troughs ranging 15–20 μg/mL lead to better outcomes of MRSA bacteremia (Kullar et al. 2012).

MRSA also causes relatively high mortality from ventilator-associated pneumonia, even when appropriate early therapy with vancomycin is administered at a dosage of 15 mg/kg every 12 h. However, because of the poor penetration of vancomycin in epithelial lining fluid, it is unlikely that this dosing schedule always achieves optimal vancomycin exposure in the lung. Vancomycin treatment failure for infections caused by MRSA strains with high MICs has prompted the American Thoracic Society and the Infectious Diseases Society of America to recommend higher vancomycin target troughs of 15–20 μg/mL for hospital-acquired, ventilator-associated, and healthcare-associated pneumonia (Rybak et al. 2009a). A prospective cohort study comparing 51 adult patients infected with MRSA with a vancomycin MIC of ≥2 with 44 patients due to MRSA with a vancomycin MIC < 2 μg/mL, response was significantly lower (62 % vs. 85 %, $P = 0.02$) and infection-related mortality was higher (24 % vs. 10 %); $P = 0.16$ in the high MIC group. High MIC ($P = 0.03$) and Acute Physiology and Chronic Health Evaluation II score ($P = 0.009$) were independent predictors of poor response in multivariate analysis. The authors concluded that high prevalence of clinical MRSA strains with elevated vancomycin MIC (2 μg/mL) requires aggressive empirical vancomycin dosing to achieve a trough greater than 15 μg/mL, and that combination or alternative therapy should be considered for invasive infections caused by these strains (Hidayat et al. 2006).

Continuous infusion of vancomycin may enhance vancomycin efficacy with the standard 30 mg/kg daily dosage, thus avoiding the need to use higher total daily dosages that could increase the risk of nephrotoxicity. This strategy is illustrated in Clinical vignette 12.1. In the case of fully susceptible pathogens with an MIC of

Clinical Vignette 12.1: TDM of Vancomycin Administered by Continuous Infusion

A 21-year-old student has developed subacute meningitis and peritonitis in the presence of a ventriculoperitoneal shunt that was placed for hydrocephaly that developed following removal of a brain tumor. He has a fever of 38.9 °C, headache, and severe abdominal pain.

His length is 187 cm and his weight is 67 kg. The cerebrospinal fluid (CSF) WBC count is $>2.0 \times 10^9$/ml and the Gram stain shows Gram positive cocci in clusters.

Empirical therapy with vancomycin is started at a loading dose of 15 mg/kg and a daily dose of 30 mg/kg/day by i.v. continuous infusion (CI). In practice, two syringes of 1 g are administered over 12 h with an infusion pump. Both *S. aureus* MSSA (MIC 1 μg/mL) and coagulase-negative staphylococci (MIC 2 μg/mL) are cultured from the CSF. The shunt is removed and a ventricular external drain is placed. Vancomycin 10 mg is given once intraventricularly.

A vancomycin serum level on Day 3 is 15 μg/mL. The daily CI vancomycin dose is increased to 2,250 mg (two syringes of 1,225 mg) and in the subsequent days target serum levels of 20 μg/mL are reached.

Because of the CI, each serum level sample is a correct one, regardless of the time of sampling, and adjustments of dosing are easily achieved in this neurosurgical department.

≤1 μg/mL, the strategy of targeting a steady-state vancomycin concentration of 15 μg/mL during continuous infusion may simultaneously enable an AUC/MIC ratio of ≥360, so that both pharmacodynamic efficacy targets may be optimized (Pea and Viale 2008). The potential benefit of CI vancomycin was assessed in ten patients treated with conventional dosing (CD) and continuous infusion (CI) vancomycin therapy in a prospective, randomized, crossover study. CD therapy consisted of 1 g of vancomycin every 12 h. CI therapy consisted of a 500 mg loading dose followed by 2 g infused over 24 h. CI and CD vancomycin therapy demonstrated equivalent pharmacodynamic activities. CI therapy was more likely to result in serum bactericidal titers that remained above 1:8 for the entire regimen. Serum drug concentration variability was observed with both treatment regimens, but to a lesser extent with CI administration (James et al. 1996). A multicenter, prospective, randomized study was designed to compare CI vancomycin (targeted plateau drug serum concentrations of 20–25 μg/mL) and CD, i.e., intermittent infusions (targeted trough drug serum concentrations of 10–15 μg/mL) in 119 critically ill patients with MRSA infections; microbiological and clinical outcomes and safety were similar. CI patients reached the targeted concentrations faster (36 ± 31 vs. 51 ± 39 h, $P = 0.029$) and fewer samples were required for treatment monitoring than with intermittent infusion patients (7.7 ± 2.2 vs. 11.8 ± 3.9 per treatment, $P < 0.0001$). The variability between patients in both the AUC_{24h} and the daily dose given over 10

days of treatment was lower with CI than with CD (variances, 14,621 vs. 53,975 mg h/L ($P=0.026$) and 414 vs. 818 g ($P=0.057$), respectively) resulting in lower costs (Wysocki et al. 2001). Loading doses of 25–30 mg/kg have been recommended for critically ill patients with MRSA infections (Rybak et al. 2009b). Recent intervention studies have shown the need for such doses to reach sufficient target levels during the first days of vancomycin treatment in intensive care patients (Saugel et al. 2013; Truong et al. 2012).

Teicoplanin: Higher Dosage Regimens

Patients with neutropenia after treatment for leukemia, burns, and the critically ill have lower trough levels after standard dosing and need higher (loading) doses of teicoplanin to achieve target trough concentrations. Therefore, the loading dose of teicoplanin should be tailored to individual neutropenic patients. Teicoplanin trough plasma concentrations were followed in 11 neutropenic patients after repeated administration of a 6 mg/kg i.v. bolus. The first three injections were given at 12-h intervals, and the rest every 24 h. Trough plasma concentrations at 48 h varied from 5.6 to 13.1 μg/mL (Gimenez et al. 1997). In a prospective study, adult patients with normal renal function previously treated for acute leukemia, and subsequently developing febrile neutropenia, received a high loading regimen (800 + 400 mg 12 h apart on day 1, 600 + 400 mg 12 h apart on day 2) followed by a high maintenance regimen (400 mg every 12 h) from day 3 on. In favorable comparison with a standard dosage group, teicoplanin C_{min} averaged ≥10 μg/mL within 24 h, and this value was achieved within 48 h in all but one patient. C_{min} at 72 h exceeded 20 μg/mL in 10 of the 22 patients (45 %) (Pea et al. 2004).

In a study in critically ill patients receiving a standard teicoplanin regimen for adults, only 4 of 14 exceeded trough serum concentrations of 10 μg/mL (Whitehouse et al. 2005). Therefore, it is advised to also use higher loading and maintenance doses and perform monitoring of trough levels (see therapeutic drug monitoring and Clinical Vignette 12.3).

Vancomycin and Teicoplanin: Therapeutic Drug Monitoring

Before the recognition of vancomycin resistance, the value of routine monitoring of serum vancomycin concentrations was often questioned (Freeman et al. 1993; Cantu et al. 1994; Moellering 1994; Saunders 1995). Such monitoring could be justified if it resulted in maximal therapeutic efficacy with minimal toxicity, but the available evidence is contradictory. It appears that higher peak serum levels do not correlate with more successful therapy, and there is no obvious association between peak levels and either oto- or nephrotoxicity (see under toxicity). For these reasons, it has been stated in the past that in patients with normal renal function receiving

standard vancomycin doses, routine monitoring of serum levels is not essential, although determination of trough levels may be considered (Saunders 1995). However, the AUC/MIC correlates with efficacy and trough levels can be used as a surrogate marker for AUC. As stated above, it has become apparent that the traditional vancomycin trough levels of 5–10 μg/mL are too low for difficult-to-treat infections caused by *S. aureus*, especially those due to strains with reduced vancomycin susceptibility. A minimum trough level of 10 μg/mL is now considered appropriate by some authors, while others advocate trough concentrations of 15–20 μg/mL to improve efficacy and avoid development of resistance (Rybak et al. 2009a, b). These guidelines state that monitoring of serum vancomycin levels should be performed in (1) patients being concomitantly treated with nephrotoxic drugs, such as aminoglycosides; (2) patients with acute renal failure being treated with infrequent vancomycin doses; (3) patients with renal impairment (including those with stable impairment in whom vancomycin doses have been accordingly adjusted); (4) patients with altered vancomycin pharmacokinetics—e.g., preterm infants, intensive care patients, patients with burns, pregnant women, patients with liver disease, and pediatric oncology patients; and (5) patients with deep-seated sepsis or those receiving higher vancomycin doses, for example in cases of penicillin G-resistant pneumococcal meningitis (Rybak et al. 2009a, b).

Serum level monitoring of teicoplanin may help to prescribe appropriate doses in patients with severe infections. This strategy is illustrated in clinical vignette 12.2.

Clinical Vignette 12.2: Infected Aortic Prosthesis Treated with Teicoplanin i.v./i.m.

A 34-year-old CEO has developed a postoperative infection of the sternal wound and subsequently of the vascular prosthesis of the thoracic aorta that was placed for an aneurysm of the aortic arch.

Cultures of the wound, pleural fluid, and several blood cultures yield *E. faecalis*, resistant to ampicillin. The MIC of teicoplanin is 0.25 μg/mL. His length is 178 cm and his weight is 70 kg.

Teicoplanin treatment is started. After a loading dose of 800 mg, a dose of 400 mg is administered i.v. once daily (OD). A trough level is 12 μg/mL.

Prolonged treatment with teicoplanin i.v. 600 mg OD in short bolus is given in an outpatient setting. During the weekends he stays at his lakeside cottage, and his general practitioner injects the teicoplanin i.m.

Renal function and trough levels are determined once weekly to assure a level of 15–20 μg/mL.

After normalization of inflammatory parameters, in particular C reactive protein (CRP) and subsequently the erythrocyte sedimentation rate, the synthetic prosthesis is removed and replaced by a bioprosthesis of the aortic arch and aortic valve and teicoplanin treatment is continued for another 6 weeks postoperatively. There has not been any recurrence of the infection.

Fluorescence polarization immunoassay (FPIA) is usually the most specific, reliable, and rapid assay. Although the product information states that trough serum concentrations should not be less than 10 μg/mL (Sanofi Aventis 2006), others advise that trough levels just prior to next dose should not be less than 20 μg/mL (MacGowan et al. 1992; Phillips and Golledge 1992; Wilson et al. 1993).

Teicoplanin has been widely used in the UK. Higher teicoplanin dosage recommendations for specific infections have not led to significant increase in the proportion of predose concentrations >20 μg/mL between 1994 and 1998 in samples sent for teicoplanin assay at the Regional Antimicrobial Reference Laboratory, Bristol, UK. A questionnaire on the use of teicoplanin and therapeutic drug monitoring (TDM) was sent to all UK National External Quality Assurance Scheme antibiotic assay users. Fewer than 25 % recommended teicoplanin TDM during routine use, the main reasons being perceived lack of toxicity and lack of evidence for the use of teicoplanin TDM. Predose concentrations <20 μg/mL were considered appropriate for treatment of bacteremia caused by methicillin-resistant *S. aureus* by 53 % of those responding. Data sheet advice was relied upon more than TDM as an indication of therapeutic dosing. Microbiologists who mainly used vancomycin tended to perform more TDM and seek higher serum concentrations when using teicoplanin than those who preferentially used teicoplanin (Darley and MacGowan 2004).

The serum concentrations of teicoplanin in 48 patients with MRSA pneumonia were monitored and compared for their clinical efficacy, investigating the significance of the mean dose administered during the initial 3 days. Teicoplanin was given at a loading dose of 400 or 800 mg on the first day, followed by maintenance doses of 200 or 400 mg. The mean initial dose (MID) over the first 3 days was calculated as: (loading dose + dose on second day + dose on third day)/3. Patients with an MID of 266.7 mg or less (400 mg for loading, 200 mg over the second and third days) did not have a trough level that exceeded 10 μg/mL at the point before the injection on the fourth day. Even in patients with hemodialysis (HD), an MID of 266.7 mg was not enough to provide a trough level of 10 μg/mL. Patients with an MID higher than 533.3 mg had significantly elevated trough levels, showing better outcomes. A multiple regression formula for predicting trough level before the fourth day of administration is given as: $0.034 + 0.030 \times (\text{MID; mg}) - 0.057 \times \text{creatinine clearance (Ccr; mL/min)}$. These findings suggest that 800 mg as an initial dose, followed by 400 mg maintenance doses over the following 2 days, makes it possible to safely attain an optimal trough level, even in the patients with HD (Sato et al. 2006).

A pilot study assessed the safety and efficacy of three-times weekly teicoplanin in the outpatient treatment of 10 acute posttraumatic osteomyelitis. Pathogens were MRSA (five patients) and MRSE (five patients). After a loading dose of 400 mg b.i.d. for 3 days, patients were treated with an i.v. dose of 1,000 mg on Mondays and Wednesdays and with a 1,200 mg dose on Fridays. Teicoplanin trough levels were maintained within a 10–20 μg/mL range. If hardware removal had been possible at enrollment, treatment was carried out for at least 4 weeks. If, on the contrary, hardware removal had not been possible, teicoplanin was administered as suppressive therapy until hardware removal. Treatment was successfully performed in 9 out of 10 patients, whereas in one patient only improvement was achieved. Side effects were not recorded (Lazzarini et al. 2002).

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Chapter 13
Clinical Pharmacodynamics of Quinolones

George L. Drusano, H.S. Heine, and A. Louie

Abstract Over the past two decades, the quinolones have been the class of anti-infective agent for which the most pharmacodynamics relationships have been elucidated. Relationships for clinical and microbiological outcomes have been developed for patients with Hospital-acquired and Community-acquired infections. Advances in the use of Monte Carlo simulation have allowed the adequacy of dose choice for specific pathogen MIC distributions to be calculated.

For the first time, other pathogens have had pharmacodynamics principles applied to therapy. This has been particularly true for the therapy of tuberculosis, both in the clinic and also with the use of preclinical tools to elucidate optimal therapy.

The therapy of select agents, such as *Bacillus anthracis* (anthrax) and *Yersinia pestis* (plague), can never be validated in man. Therefore, use of pharmacodynamics principles have become a mainstay for the design of animal studies for the "Two Animal Rule," which permit the issuance of a claim for an indication for these select agents by the FDA.

Keywords Quinolones • Pharmacodynamics • Hospital-acquired infections • Community-acquired infections • Tuberculosis • Anthrax • Plague

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Introduction

In this chapter, the clinical pharmacodynamics of quinolone use is examined. Fluoroquinolones have had more effort directed to elucidating exposure–response relationships than any other class of antimicrobial agent. We look mostly at different indications but concentrate on community infection use and use in the intensive care unit, particularly for nosocomial pneumonia. We briefly examine their use in tuberculosis. At the end, we examine indications for which there cannot be clinical trials, the therapy of select agent infections such as anthrax and plague, where fluoroquinolones have proven to be central to therapy.

PK/PD Indices Linked to Outcomes for Quinolones

The first data relating fluoroquinolone exposure and its link to outcome was an in vitro study by Blazer et al. (1987). In this hollow fiber evaluation, the authors concluded that peak/MIC ratio was the dynamically linked index, but this is likely because in this study there was confounding between cell kill and suppression of resistance. Leggett et al. examined this issue in mouse thigh and lung models and came to the conclusion that AUC/MIC ratio was the dynamically linked index, possibly because resistance emergence was not sought (Leggett et al. 1990). Drusano and colleagues used a rat sepsis model with a very dense bacterial burden (circa 10^9 cells) and were able to show that sometimes it appeared that peak/MIC ratio was linked to outcome and sometimes it appeared as though AUC/MIC ratio was the linked index (Drusano et al. 1993). Again, this is highly likely to be due to the preexistence of resistant mutants in the population due to the large baseline burden, highlighting the confounding between the twin endpoints of cell kill and suppression of resistant mutant amplification. So, it can be said that for quinolones, both AUC/MIC ratio AND peak/MIC ratio may appear to be linked to outcome.

Therapy of ICU Infections

The first robust examination of fluoroquinolone pharmacodynamics was published first by Peloquin et al. (1989) and subsequently by Forrest et al. (1993a) from the same laboratory, with considerable overlap of patients. The most notable finding of the Peloquin paper (Peloquin et al. 1989) was the rate of resistance emergence seen among isolates of *Pseudomonas aeruginosa* to ciprofloxacin. These data were collected early in the development of intravenously administered ciprofloxacin. The dose employed was 200–300 mg IV every 12 h. There were 10 patients with *Pseudomonas aeruginosa* pneumonia. Of these, seven emerged resistant to ciprofloxacin during the course of therapy. There were three other patients with either

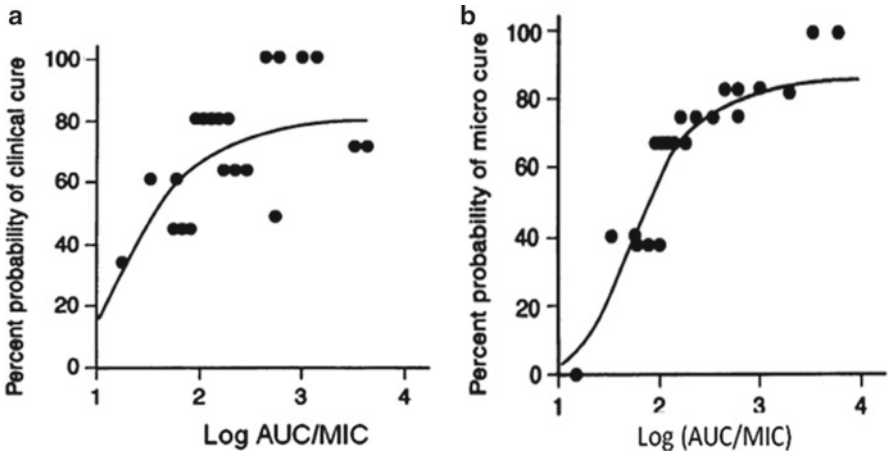

Fig. 13.1 Relationship between ciprofloxacin AUC/MIC ratio and the probability of clinical (Panel **a**) and microbiological (Panel **b**) success

Pseudomonas bronchiectasis or empyema. Of these patients, all had resistance emergence. Therefore, the rate of resistance was 7/10 (70 %) or 10/13 (77 %). Such a resistance rate is insupportable in the clinic and points to the inadequate dose of drug. Many years later, Jumbe et al. (2003) demonstrated in a mouse model that suppression of resistance emergence required a substantial exposure to fluoroquinolones, with an AUC/MIC ratio of 157 being the exposure required for a strain of Pseudomonas. Monte Carlo simulation from another study by Forrest et al. (1993b) demonstrated that a 200-mg IV dose every 12 h attained this target approximately 25 % of the time, fitting quite nicely with the 70–77 % rate of resistance emergence.

The Forrest paper (Forrest et al. 1993a) was a retrospective analysis of multiple clinical trials of ciprofloxacin conducted at a single site. Overall, there were 74 patients in the total population with microbiology and pharmacokinetic data. For clinical outcome, 66 patients were evaluated (46 cured/20 failed) with 64 of the 66 evaluated in the time-to-event analysis. For microbiologic outcome, 64 patients were analyzed (42 cured/22 failed). Forty-one were treated with 200 mg IV Q12h, with 8, 1, and 24 patients receiving 300 mg IV Q12h, 400 mg IV Q12h, and 400 mg IV Q8h, respectively. Fifty-eight patients had a lower respiratory tract infection, nine had skin/skin structure infection, four had primary bacteremia, and three had a urinary tract infection. *Pseudomonas aeruginosa* was recovered from 25/74 patients.

Analysis demonstrated that there was a highly significant relationship between AUC/MIC ratio and both clinical and microbiological outcome. These are displayed below in Fig. 13.1, Panels a and b.

What is clear is that there was a high failure rate among these patients. Substantial exposure is required to have a high likelihood of success for either endpoint. Part of this is because of the severity of the infection in the subset of patients with lower respiratory tract infection. Nonetheless, this was a groundbreaking paper showing

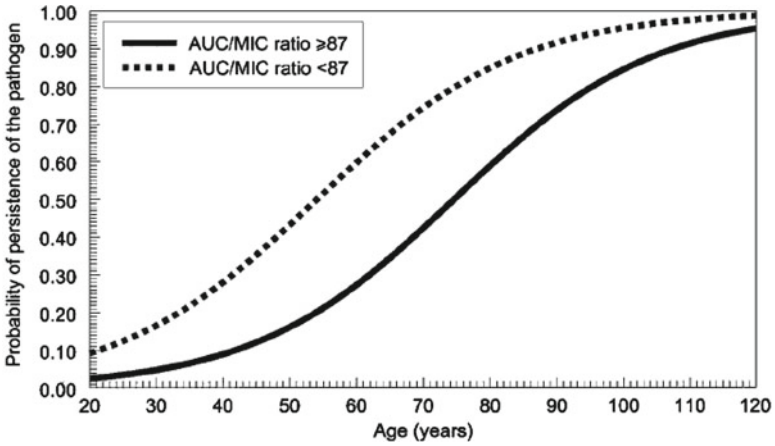

Fig. 13.2 Probability of pathogen persistence as a function of age and whether or not an AUC/MIC ratio of 87.0 was attained

that it is possible to identify exposure–response relationships in the clinic. It should be noted that there is no other area of medicine where there has been this number of successful attempts at constructing exposure–response relationships. Antimicrobial chemotherapy is unique because of the ability to normalize drug exposure to a measure of potency of the drug for the pathogen in question (the MIC). Other areas currently have no method to identify the between-patient variability of the affinity of binding of the drug for the receptor being targeted.

A number of years later, Drusano et al. (2004) performed a prospective evaluation of levofloxacin in patients with hospital-acquired bacterial pneumonia. In this clinical trial, 58 patients had plasma pharmacokinetics determined for levofloxacin administered as a 750-mg IV dose over a 1.5-h infusion once daily. Of these, 47 patients also had an organism identified and also had a microbiological outcome assessed. In a multivariate logistic regression, levofloxacin AUC/MIC ratio was defined as significantly influencing the probability of a good outcome along with the age of the patient. In this population, the median age was 53 years, which is considerably younger than the population studied by Forrest et al. (1993a). The median APACHE II score was 16 with a range of 5–28. Of the 47 patients, 46 had residence in the ICU. There were 83 pathogens recovered, with 8 *Pseudomonas aeruginosa*, 19 other Gram-negative Enterobacteriaceae, 5 other nonfermentative Gram-negative bacilli, and 17 *Staphylococcus aureus*.

Classification and regression tree (CART) analysis identified a breakpoint for microbiological outcome in AUC/MIC ratio of >87.0 and ≤109.8. It should be noted that there were no patients who had an AUC/MIC ratio between these figures and, therefore, the breakpoint may be rationally attached to the low end, the middle (98.5), or the high end. The authors chose the low end of an AUC/MIC ratio of 87.0. Figure 13.2 shows the relationship for the probability of a good microbiological outcome, as influenced by AUC/MIC ratio and age.

Table 13.1 Model performance

Characteristic, parameter	Eradication of pathogen	Persistence of pathogen
Age <67 years		
AUC:MIC ratio greater or equal to breakpoint	26	1
Sensitivity	26/29 (89.6)	
Specificity	1/2 (50.0)	
Positive predictive value (predicting eradication)	26/27 (96.3)	
Negative predictive value (predicting persistence)	1/4 (25.0)	
Age ≥67 years		
AUC:MIC ratio less than breakpoint	0	3
AUC:MIC ratio greater or equal to breakpoint	10	3
Sensitivity	10/10 (100.0)	
Specificity	3/6 (50)	
Positive predictive value (predicting eradication)	10/12 (76.9)	
Negative predictive value (predicting persistence)	3/3 (100.0)	

Note: Data are no. (%) of patients

CART analysis defined an age of 67 as the breakpoint. In Table 13.1, we show the model performance for patients above and below the breakpoints of 87.0 for AUC/MIC ratio and 67 years for age.

It is obvious by inspection that patients younger than 67 eradicate their pathogen irrespective of the AUC/MIC ratio achieved, whereas older patients really need to achieve the AUC/MIC breakpoint value to eradicate their infecting pathogen.

The question arises as to the importance of such information. Clearly, it can be demonstrated that the AUC/MIC ratio has an important impact on both clinical and microbiological outcome when both the Forrest and Drusano studies are examined. In order to make this data informative to clinicians, the levofloxacin study performed a Monte Carlo simulation and examined the target attainment for different MIC values for both *Pseudomonas aeruginosa* and *Enterobacter cloacae*, the two most common Gram-negative causes of nosocomial pneumonia. This is shown in Fig. 13.3.

Overall, we can identify the utility of a drug dose by taking the expectation over the MIC distribution with respect to the target attainments at each MIC value. For a target of an AUC/MIC ratio of 87.0, the expectation for the 750-mg levofloxacin dose is 72 % for *Pseudomonas aeruginosa* and is 92 % for *Enterobacter cloacae*. If one uses 109.8 as the target, these values are 69 % and 89 %, respectively. Clearly levofloxacin, at the 750-mg IV dose is adequate for *Enterobacter cloacae*, but not for *Pseudomonas aeruginosa*. In this study, if Pseudomonas was suspected or recovered from the patient, the attending clinician could add a β-lactam agent. West et al. (2003) report from the overall study that there was no instance of resistance emergence with *Pseudomonas aeruginosa* when levofloxacin was combined with a β-lactam. This is consistent with in vitro data generated by Louie et al. (2010), where the combination of meropenem plus levofloxacin suppressed resistance, even in a strain overexpressed for *mex*AB. This puts forth the hypothesis that Pseudomonas

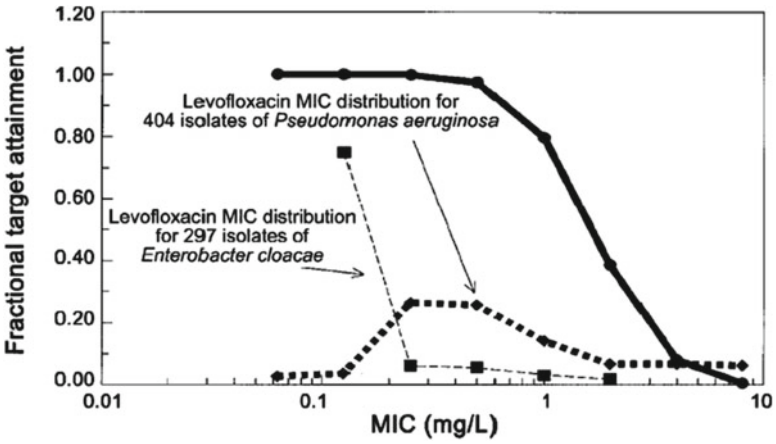

Fig. 13.3 Target attainment (AUC/MIC = 87.0) for a daily levofloxacin dose of 750 mg for *Pseudomonas aeruginosa* and *Enterobacter cloacae*

may require combination therapy to optimize outcome, particularly in circumstances where there are large bacterial burdens, such as in patients with HABP (Hospital-Acquired Bacterial Pneumonia) or VABP (Ventilator-Associated Bacterial Pneumonia). Performing the pharmacodynamics for combination chemotherapy is the next challenge for antimicrobial pharmacodynamics.

Therapy of Community-Acquired Infections

The first study to delineate the relationship between fluoroquinolone exposure and outcome in community-acquired infections was published by Preston et al. in the *Journal of the American Medical Association* (Preston et al. 1998a), where 500 mg of levofloxacin administered both IV and by mouth was employed for the therapy of community lower respiratory tract infection, skin and skin structure infection, and sinusitis and urinary tract infection. There were 313 patients enrolled, of whom 272 were included in the pharmacokinetic analysis. Of those with plasma concentration–time data, 134/272 also had an infection site identified along with a recovered pathogen with an MIC value for levofloxacin. Of the 134 patients evaluable for clinical outcome, 116 were also evaluable for microbiological outcome. The population pharmacokinetics was published elsewhere (Preston et al. 1998b). Multiple independent variables, including Peak/MIC ratio, AUC/MIC ratio and time > MIC were examined for impact on clinical and microbiological outcomes (dependent variables) by logistic regression analysis. All pharmacokinetic indices had a significant impact on the likelihood of a good clinical outcome. The final model included the PD index Peak/MIC ratio as well as site of infection. The final model ($p < 0.001$) is shown in Table 13.2; the logistic functions for Peak/MIC ratio and AUC/MIC ratio are displayed in Fig. 13.4, Panels a (p, 0.001) and b ($p < 0.006$).

Table 13.2 Logistic regression analysis examining clinical outcome ($n = 134$); final model

Covariate	Estimate	Standard error
Constant	0.970	0.876
Peak/MIC ratio	0.140	0.064
Site[a]		
Pulmonary	0	–
Skin/skin structure	−1.06	0.876
Urinary tract	36.516	>100

[a]For categorical variables, the estimate is added to the logit

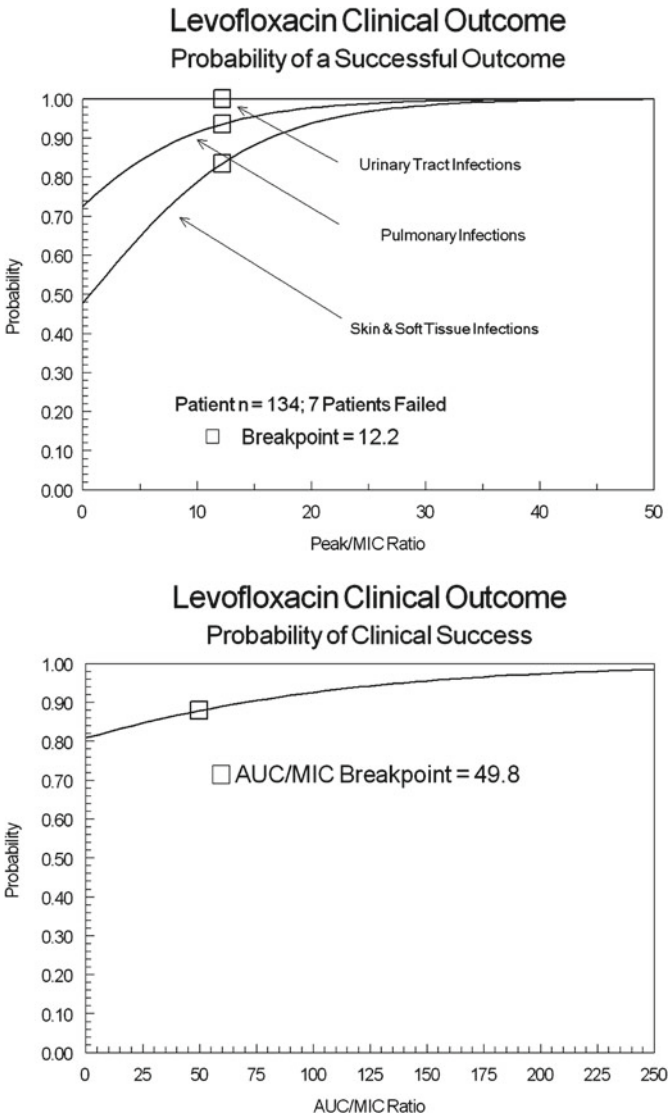

Fig. 13.4 Logistic functions for clinical outcome: Panel (**a**)—peak/MIC ratio plus site of infection; Panel (**b**)—AUC/MIC ratio

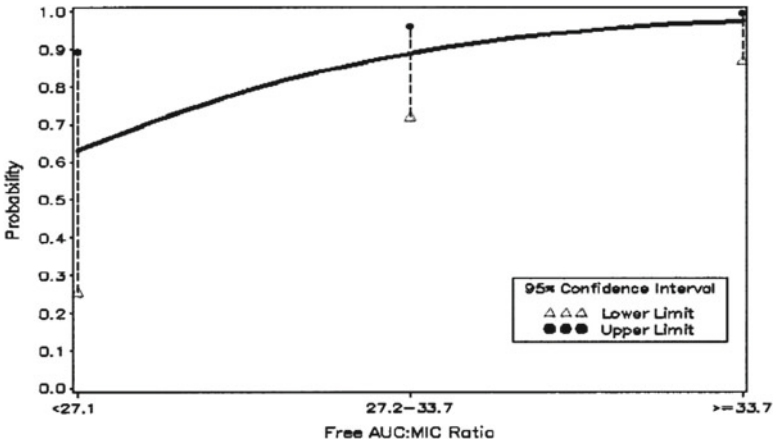

Fig. 13.5 Probability of pneumococcal eradication by a fluoroquinolone

The breakpoints for Peak/MIC ratio (12.2) and AUC/MIC ratio (49.8) were determined by CART analysis as indicated above. It is clear that the AUC/MIC breakpoint of approximately 50 mediates an 88 % probability of a good clinical response, which is quite different from the breakpoint of 125 seen in the Forrest data (Forrest et al. 1993a). One question of importance not answerable from this data set is the breakpoint for patients with pneumococcal pneumonia. Only one patient with pneumococcal pneumonia failed.

Ambrose and colleagues addressed this important question in a study of gatifloxacin versus levofloxacin in community-acquired pneumonia (Ambrose et al. 2001). There was a mixture of patients (circa 50:50) with CABP (Community-Acquired Bacterial Pneumonia) and AECB (Acute Exacerbation of Chronic Bronchitis) and the total patient number was 58. Again, CART analysis was employed to identify a breakpoint value. For this data set, the value was a free drug AUC/MIC ratio of 27.2–33.7. This is displayed in Fig. 13.5 ($p = 0.013$).

This finding was very important because it demonstrated that the likelihood of a good microbiological outcome was very high when nonciprofloxacin fluoroquinolones were employed for the therapy of pneumococcal respiratory infection.

Nonetheless, the number of patients contributing to this analysis was small and there was mixture of patients with pneumonia and AECB. More recently, Bhavnani et al. (2008) examined a larger number of patients ($n = 98$), all of whom had *Streptococcus pneumonia* CABP. These patients came from ten separate trials. Importantly, these data allowed identification of an exposure response relationship for both clinical and microbiological outcome. Again, the clearest results were produced by CART analysis. The identified breakpoint of a free drug AUC/MIC ratio of 33.8 is virtually indistinguishable from the earlier pneumococcal breakpoint

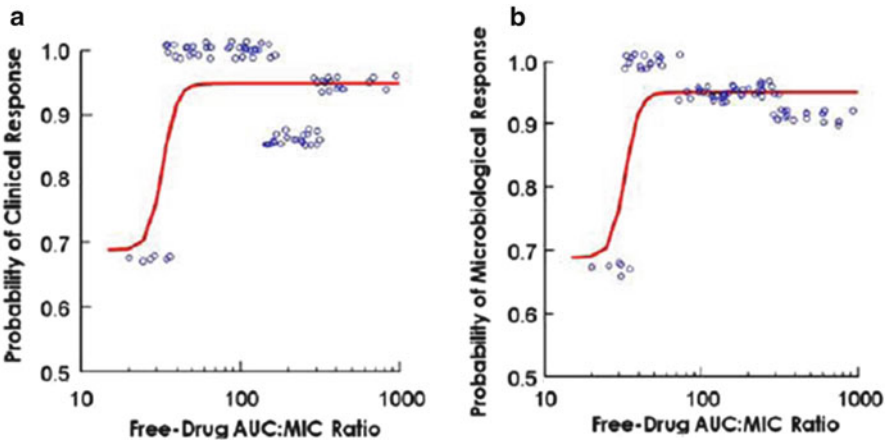

Fig. 13.6 Relationship between probability of clinical (**a**) and microbiologic (**b**) success versus the free-drug AUC/MIC ratio in patients with CABP arising from *S. pneumoniae* who received treatment with quinolones

of 33.7 identified by Ambrose et al. (2001). The likelihood of a good clinical outcome was 67 % if the breakpoint was not achieved and was 95 % if the breakpoint was achieved. It should be noted that the majority of these patients were treated in an outpatient setting. This means that the majority had a low Pneumonia Severity Index (PSI) score. Nonetheless, it is impressive that the breakpoint was discernible in this circumstance and that optimal drug exposure mediated an absolute 28 % improvement in clinical outcome. The authors also fit a Hill model to the data that allows graphic presentation of the data. This is shown in Fig. 13.6 ($p = 0.03$).

The issue of low PSI scores and outcome is highly important if quinolones are to be thought of as adequate agents for the therapy of serious CABP caused by the pneumococcus. Dunbar et al. (2003) examined this issue in a randomized, double-blind trial of CAPB with levofloxacin in which 500 mg daily for 10 days was compared to 750 mg daily for 5 days. In Table 13.3, the data for clinical response are shown stratified for the Pneumonia Severity Index. There were 198 patients in the high-dose short-course group and 192 patients in the standard group. There were very few patients in the stratum of PSI class V patients. However, examining strata III and IV demonstrates that clinical responses were quite acceptable for both groups and they were not statistically different. It is important to note, however, that only 42 isolates of *Streptococcus pneumonia* were recovered, 22 in the high-dose short-course group and 20 in the standard therapy group. Response rates were 90.9 % and 90.0 %, respectively, with an overall response to the fluoroquinolone of 90.5 %. The overall message, however, is that a fluoroquinolone was able to provide highly acceptable response rates for seriously ill patients infected with *S. pneumoniae*.

Table 13.3 Clinical success rates for the clinically evaluable population at the 7–14-day posttherapy visit, according to the Pneumonia Severity Index (PSI) score

	n/N (%)[a]		
Patient category	750-mg group ($n=198$)	500-mg group[c] ($n=192$)	95 % CI[d]
Evaluable patients	183/198 (92.4)	175/192 (91.1)	−7.0 to 4.4
Stratum I[e]			
Total	69/76 (90.8)	73/86 (84.9)	−16.5 to 4.7
PSI class III[f]	44/49 (89.8)	44/51 (86.3)	−17.2 to 10.2
PSI class IV[g]	25/27 (92.6)	27/32 (84.4)	−26.1 to 9.6
PSI class V[h]	0/0 (0.0)	2/3 (66.7)	Not applicable
Stratum II[i]	114/122 (93.4)	102/106 (96.2)	−3.4 to 9.0

[a]Number of patients in the category with clinically successful treatment/no. of patients in the category (%)
[b]Levofloxacin, 750 mg q.d. iv or po for 5 days
[c]Levofloxacin, 500 mg q.d. iv or po for 10 days
[d]Two-sided 95 % CI around the difference (10-day levofloxacin regimen minus 5-day levofloxacin regimen)
[e]PSI classes III, IV, and V combined
[f]PSI score, 71–90
[g]PSI score, 91–130
[h]PSI score, >130
[i]PSI classes I and II combined

Quinolones for Therapy of *Mycobacterium tuberculosis*

One of the newer uses for quinolones comes in the therapy of *Mycobacterium tuberculosis*, especially MDR-TB. Earlier, the in vitro activity of second-generation fluoroquinolones such as ciprofloxacin for TB was appreciated. However, the relative lack of potency was not appreciated (Gumbo et al. 2005). In many places, such as the Philippines, rapid resistance emergence was seen, as ciprofloxacin became the mainstay of MDR-TB regimens. In the Philippines, quinolone resistance increased from 10.5 % in 1989 through 1994 to 51.4 % from 1995 through 2000 (Grimaldo et al. 2001).

Given the lack of potent therapy for patients infected with MDR-TB, and looking to shorten the course of therapy, much interest devolved upon identification of an optimal choice among the possible agents. An innovative trial for examining this issue was performed by Rustomjee et al. (2008) and was dubbed the Oflotub trial. One of the key components of the trial was the use of Serial Sputum Colony Counts (SSCC) for the first 8 weeks and their proper analysis employing mixed effects model techniques by Geraint Davies. Four groups were analyzed (1) without a fluoroquinolone in the first 8 weeks (control), (2) with ofloxacin, (3) with moxifloxacin, and (4) with gatifloxacin.

While moxifloxacin was the best overall, gatifloxacin performed almost as well. Interestingly, ofloxacin did not clear the sputum any differently from the control

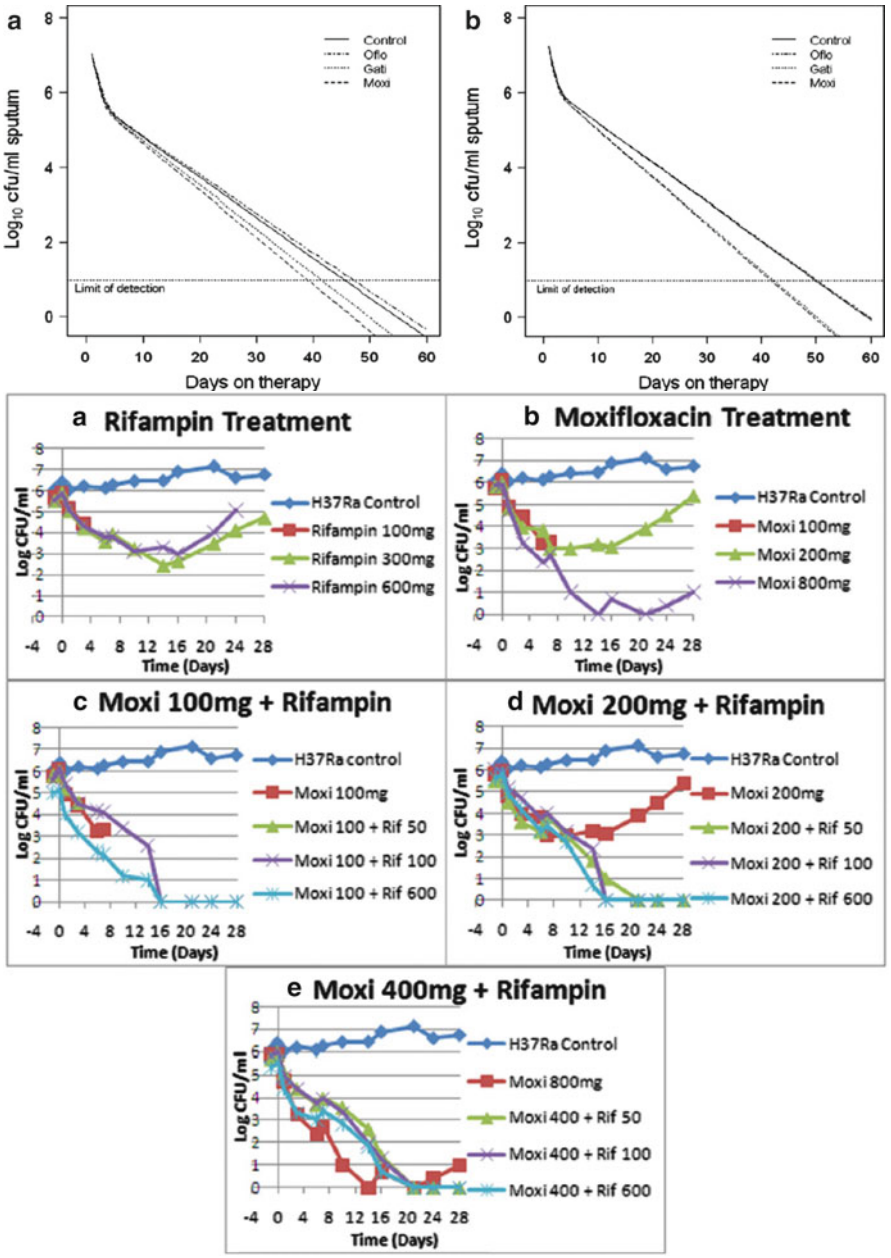

Fig. 13.7 Fitted biexponential curves for the four treatment series using the nonlinear mixed effects model (**a**) before adjustment for covariates and (**b**) after adjustment for covariates (age, HIV positivity, and radiographic disease extent)

regimen. The overall rates of clearance by regimen are displayed in Fig. 13.7, Panels a (without covariate adjustment) and b (with adjustment).

This trial is extremely important less for its outcome and more as a blueprint for evaluation of new agents in a manner most likely to generate the kind of information that will lead to robust choices among regimens by examining rates of clearance.

More recently, the issue of employing the combination of moxifloxacin plus rifampin was explored as a way of potentially shortening over duration of therapy. This combination was examined in the hollow fiber infection model. In the first evaluation (Drusano et al. 2010), the combination was evaluated against both Log-phase organisms as well as against nonreplicative persister (NRP)-phase organisms.

It should be recognized that NRP-phase organisms have very low turnover rates. Consequently, resistance emergence is inhibited to some degree because of the very slow doubling time. Even though *M. tuberculosis* has a relatively long doubling time in Log-phase, resistance emergence can be seen for all drugs evaluated in the hollow fiber system (Gumbo et al. 2004, 2007a, b). These issues are important because the actual interaction of the two drugs (moxifloxacin and rifampin) with regard to cell kill are difficult to sort out in Log-phase growth, as antagonism is confounded with resistance emergence. However, because resistance emergence is functionally halted in NRP-phase growth, one can observe the drug interaction's impact on kill rate directly.

When examining Fig. 13.8 (Panels 1 and 2), it is clear that combination therapy suppresses all resistance emergence in this experiment when the organism is in Log-phase growth. All monotherapy arms have resistance emergence during the course of the experiment, rendering a judgment on the drug interaction (synergy, additivity, antagonism) impossible.

When the same sort of experiment is done on the organism when in NRP phase, the outcome is quite different. This is shown below in Fig. 13.9. Here, as there is no resistance amplification, it is straightforward to see the impact of the drug combination on cell kill. There is a mild, but definite and statistically significant antagonism that becomes manifest, resulting in slower cell kill. This can be demonstrated in a Kaplan–Meier analysis, where, for the Log-phase organism, time-to-resistance emergence is significantly different between mono- and combination-therapy, while in NRP phase, time-to-3 Log kill is significantly longer for the combination relative to monotherapy. This is demonstrated in Fig. 13.10, Panels a and b. Consequently, while there is excellent suppression of emergence of resistance with this regimen (Log phase), it comes at a cost of the presence of mild antagonism of kill rate (NRP phase), indicating that this combination, while a welcome addition to the armamentarium, is unlikely to have a major impact on therapy duration.

Emergence of resistance is due to a number of major causes. While inadequacy of the therapeutic regimen is often cited, it is likely that nonadherence is the most important cause of resistance emergence. Directly Observed Therapy Short course (DOTS) programs have been implemented in response to this realization. In many of these programs, therapy is suspended for the weekend because of the difficulty of reaching everyone 7 out of 7 days. Generally, this "regimen skip" is relatively

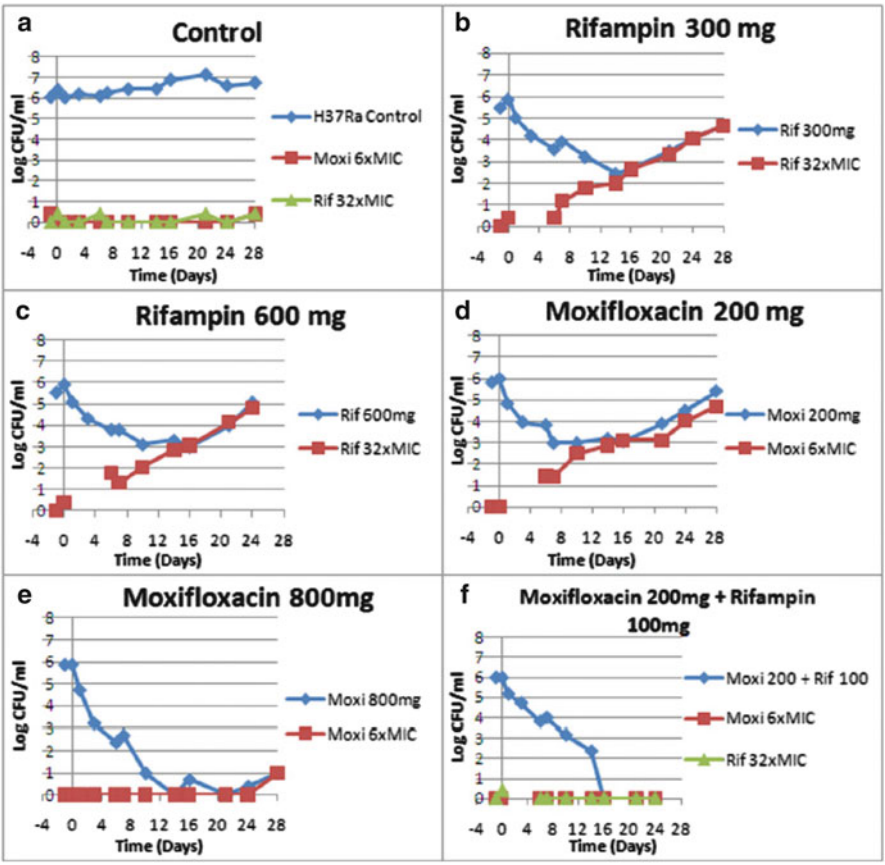

Fig. 13.8 First panel—effects of moxifloxacin alone and in combination on log-phase *M. tuberculosis* H37Ra. Second panel—emergence of resistance during drug administration

inconsequential because drugs like rifampin and isoniazid are well matched with respect to their half lives. However, moxifloxacin and rifampin are not well matched. In addition, moxifloxacin, as a fluoroquinolone, induces error-prone replication (O'Sullivan et al. 2008). In an in vitro hollow fiber experiment, the impact of the drug holiday, combined with pharmacokinetic mismatch and induction of error-prone replication was investigated (Drusano et al. 2011). In a replicated experiment, the 5 of 7 day active therapy allowed resistance emergence, while the 7 of 7 day active therapy did not. It should be realized that the actual drug exposures were important, as the mean ± 1 standard deviation of moxifloxacin plus rifampin drug exposures were each evaluated and it was only with the lowest exposure that resistance emergence was seen in the 5/7 day therapy group. With a large number of patients treated, a substantial minority (15.8 %) will develop such low exposures. Consequently, when there is great disparity of clearance and half-life between

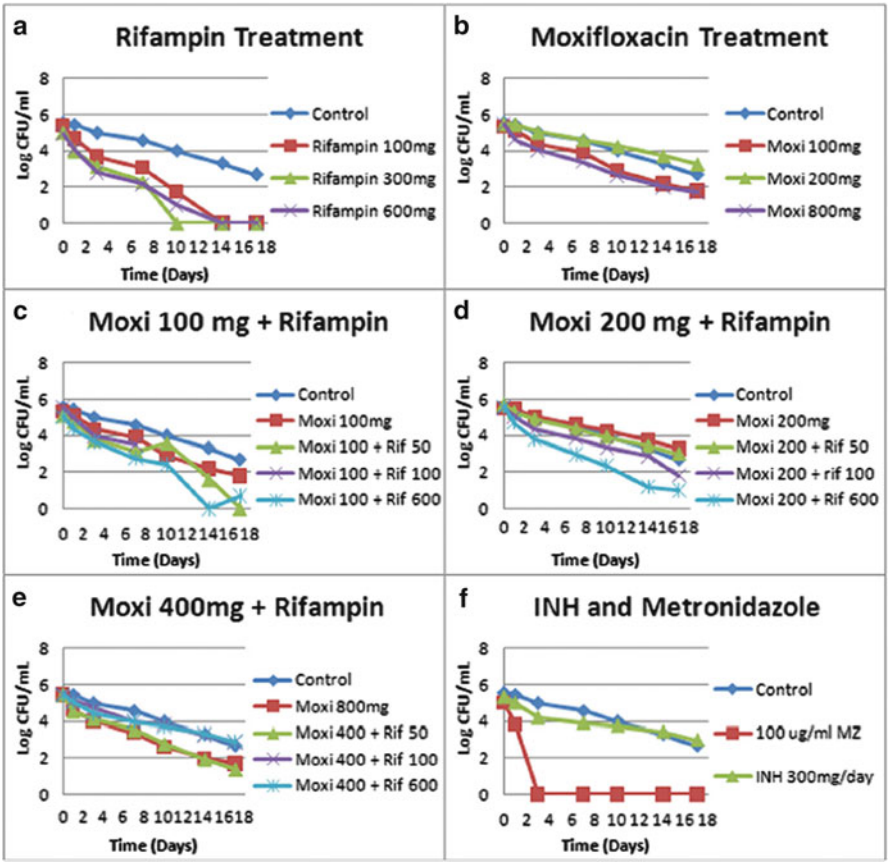

Fig. 13.9 Effects of moxifloxacin alone and in combination on NRP phase (Wayne-Hayes level II anaerobiosis) *M. tuberculosis* H37Ra

agents and when error-prone replication is operative, lower drug exposures may still result in emergence of resistance despite combination therapy. Drug holidays as part of DOTS programs need to be evaluated carefully, especially when one drug (the fluoroquinolone in this instance) induces error-prone polymerases and hence error-prone replication (Fig. 13.11).

Quinolones for the Therapy of Select Agents Such as Anthrax and Plague

One area where clinical trials simply cannot be performed on the basis of number of cases recognized per year and ethics is the therapy of patients infected with select agents, such as *Bacillus anthracis* and *Yersinia pestis*. Consequently, choice of drug,

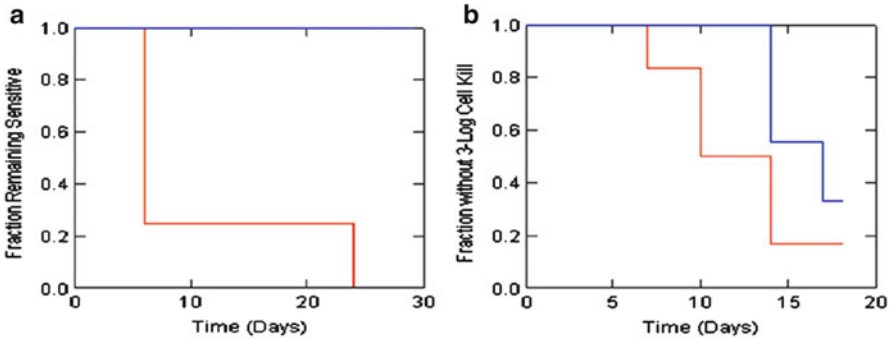

Fig. 13.10 Panel (**a**)—Times to resistance emergence in *M. tuberculosis* H37Ra for single versus combination chemotherapy. The difference is significant ($p = 0.0006$; Breslow–Gehan test). *Red* is monotherapy arms. *Blue* is combination therapy arms. Panel (**b**)—Times to achievement of a 3-log kill of *M. tuberculosis* H37Ra cells in the NRP phase for single versus combination chemotherapy. The difference is significant ($p = 0.042$; Breslow–Gehan test). *Red* is monotherapy arms. *Blue* is combination therapy arms

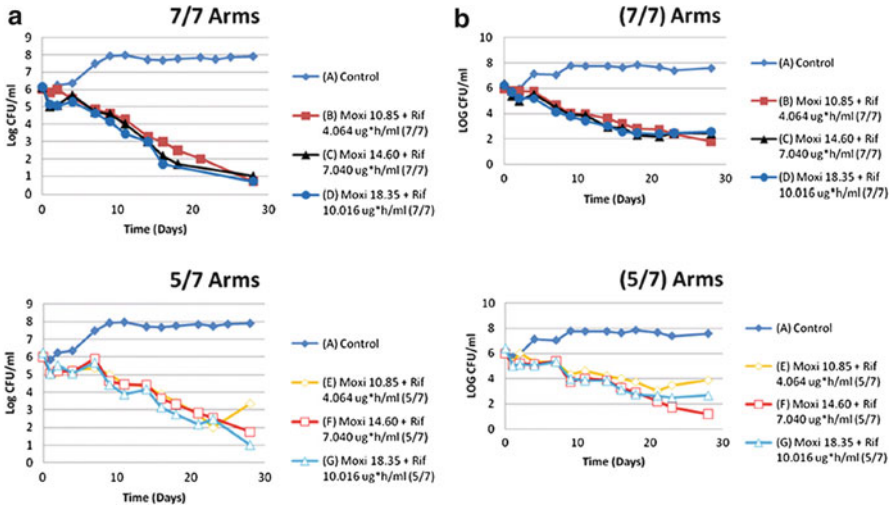

Fig. 13.11 (**a**) First trial of moxifloxacin-plus-rifampin 7/7-day versus 5/7-day regimens. The AUC exposures in the symbol keys are the free $AUC_{24\,h}$ that were infused into that particular hollow fiber system experimental arm on the days that the drugs were administered. (**b**) Second trial of moxifloxacin-plus-rifampin 7/7-day versus 5/7-day regimens. The AUC exposures in the figure legends are the free $AUC_{24\,h}$ that were infused into that particular hollow fiber system experimental arm on the days that the drugs were administered. There is regrowth between day 23 and day 28, specifically in the lowest exposure group (arm E) in the experimental arm where the drugs were administered 5/7days. There was a 2.25 Log_{10} (CFU/mL) difference between arms B and E, attributable to difference in administration schedule and weekly drug exposures. In arm E, colonies recovered had a wild-type MIC for rifampin (0.03 mg/L), but increased fourfold for moxifloxacin (0.25–1.0 mg/L)

dose, and schedule can only be informed by in vitro and animal models of infection (Heine et al. 2007a; Deziel et al. 2005). In this section, we examine the central place that fluoroquinolones have come to play for these agents of bioterrorism. Indeed, in both instances, these drugs have become the drugs of choice, surpassing older agents such as doxycycline and streptomycin.

Bacillus anthracis

The first pharmacodynamic evaluation for *Bacillus anthracis* was for the drug levofloxacin (Deziel et al. 2005). A number of such studies have been performed (Heine et al. 2007a; Deziel et al. 2005; Kao et al. 2006; Ambrose et al. 2007; Drusano et al. 2008; 2009). The first issue that needs to be addressed is that there will NEVER be a human validation study. Consequently, we can only evaluate the activity of human antibiotic exposure against select agents such as *B. anthracis* and *Y. pestis* by employing in vitro pharmacodynamic infection models in which the human half-life of an antibiotic is simulated. For animals, the half-life is often highly discordant from the human half-life and in order to properly interpret the data, the animal concentration–time profile needs to be "humanized" (i.e., administered several times per dosing interval with decreasing exposures to achieve near the same peak concentration and AUC_{0-24} as is seen in man). One of the critical issues that needs to be understood is the pathophysiology of *Bacillus anthracis* infection in the mouse model (Heine et al. 2007a). After aerosol challenge, spores (heat shock resistant) can be recovered from the lungs. Early on, there is some germination of spores to vegetative phase and subsequent bacteremia. This is followed around 36 h by vegetative-phase organisms recovered from mediastinum and spleen, indicating the spores disseminated quite early in the process. The time course of organisms in lung, mediastinum, blood, and spleen is shown in Fig. 13.12.

Deziel et al. (2005) examined levofloxacin in a hollow fiber infection model, testing the efficacy of levofloxacin against a spore-competent *B. anthracis* isolate as well as a spore-negative isogenic mutant and examined the impact of simulated human and animal half-life in the efficacy of the regimen.

In Fig. 13.13, the difference in animal and human profiles (half-lives of circa 7 h for man and 2.0 h for mice) are seen (Panel a) and the microbiological impact of human versus animal PK is demonstrated in Panel b. Differing hollow fiber system AUC values with animal PK never sterilize the system and with AUC values < 500 mg h/L, resistance emergence is seen. In contrast, the human profile rapidly sterilizes the system without resistance emergence. The inability of even very large exposures to levofloxacin with a murine profile to sterilize the system caused us to explore the difference in response between the parent spore-competent isolate and its isogenic spore-negative mutant (see Fig. 13.14).

In Fig. 13.14 (upper panel), a human PK profile sterilizes the system by 72 h, while the animal profile allows a cycle of kill and regrowth in each 24 h interval for

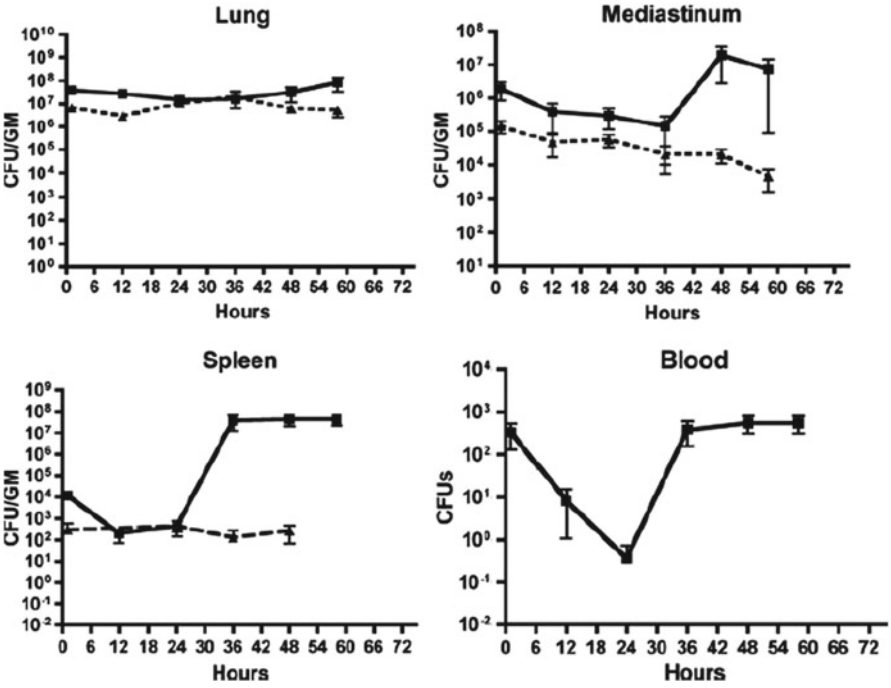

Fig. 13.12 Bacterial load disease history of *B. anthracis* (Ames) in mouse tissues after aerosol challenge. Tissues were collected from BALB/c mice at different time points postanthrax aerosol challenge ($n=6$). *Filled square*, total CFU; *filled triangle*, spores. *GM* gram of tissue

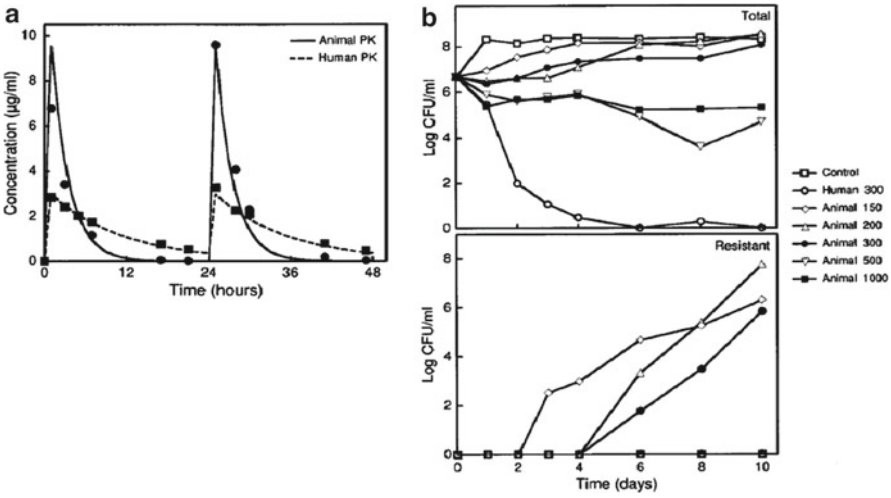

Fig. 13.13 In Panel (**a**), the human and animal (mouse and nonhuman primate have approximately the same profile) levofloxacin concentration–time profiles are shown. In Panel (**b**), *upper*, the impact of differing AUC is shown as well as the impact of human vs. animal PK. The *bottom* shows resistance emergence with low animal AUC values (<500 mg h/L)

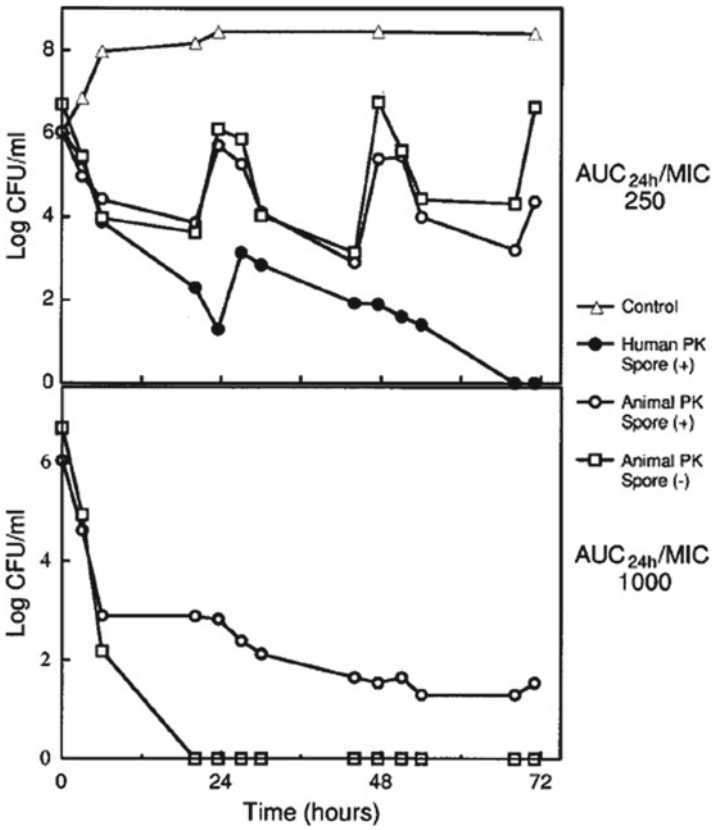

Fig. 13.14 Bacterial killing and regrowth and the role of sporulation in treatment regimens simulating human and animal pharmacokinetics. (*Upper panel*) Results obtained with daily treatment regimens with an AUC24/MIC of 250. The simulated human exposure sterilized the culture within 72 h. With animal pharmacokinetics, a cycle of killing and regrowth was seen during each 24-h dosing interval. Similar results were seen with wild-type and sporulation-negative organisms. (*Lower panel*) A simulated animal exposure at an AUC24/MIC of 1,000 rapidly sterilized the cultures of the spore-negative [spore (−)] organisms but not cultures of spore-positive [spore (+)] bacteria

both spore (+) and spore (−) isolates (AUC/MIC = 250). When a very large exposure (AUC/MIC = 1,000) is employed with an animal PK profile, the spore-negative mutant is eradicated in less than 24 h, while the spore (+) isolate persists. This demonstrates the central role of the spore form in response to chemotherapy for *B. anthracis*.

These investigators then moved to validate the in vitro findings in animal models [mouse (Deziel et al. 2005) and nonhuman primate (Kao et al. 2006)]. Both animals have levofloxacin half-lives of about 2.0 h. In the mouse model, the dosing of levofloxacin was found to have a major effect on the protectivity of the regimen. Therapy

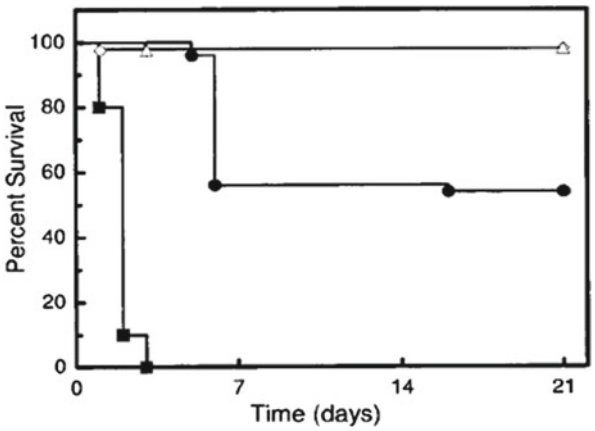

Fig. 13.15 Effect of dose schedule on efficacy of levofloxacin in an in vivo mouse model of inhalational anthrax. Dosing at 12- and 6-h intervals (*triangles* and *diamonds*, respectively) conferred virtually complete protection (one death in each treatment group [the data curves overlap]), whereas significantly more of the animals treated once daily (*circles*) died (stratified Kaplan–Meier analysis—$p < 0.000001$). All untreated control animals (*squares*) died within 3 days

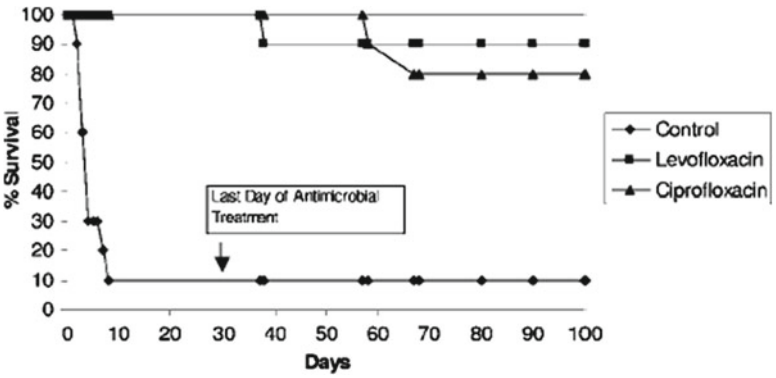

Fig. 13.16 Percent survival of rhesus monkeys over the 100-day observation period. Day 1 was the day of *B. anthracis* aerosol challenge. Antimicrobial treatment occurred from day 2 to day 31. Each treatment group was composed of five male and five female monkeys

was initiated 24-h post challenge. The more frequent dosing schedules (Q6h and Q12h) provided near-complete protection (one animal per group succumbed), while giving the whole daily dose once (AUC_{0-24} matched in all groups) resulted in a highly significant increase in mortality (see below, Fig. 13.15).

Kao et al. (2006) examined levofloxacin and ciprofloxacin for nonhuman primates that had been challenged 24 h prior to therapy with the Ames strain of *Bacillus anthracis*. Ciprofloxacin was administered twice daily, while levofloxacin was administered in a humanized fashion meant to mimic once daily dosing in man.

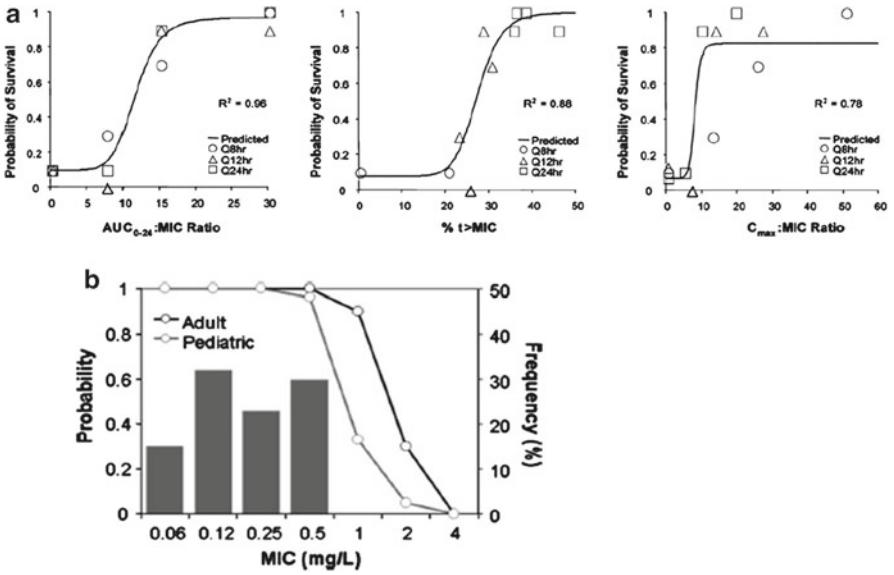

Fig. 13.17 (**a**) Relationship between three PK–PD measures (AUC0–24/MIC ratio, *C*max/MIC ratio, and %T>MIC) and survival of neutrophil replete mice challenged with aerosolized *B. anthracis* (Ames strain) after 21 days of therapy with gatifloxacin. Observed data for individual regimens stratified by dosing regimen are shown by different symbols. Q8hr, Q12hr, and Q24hr, every 8, 12, and 24 h, respectively. (**b**) Probabilities of attaining the PK–PD target ($AUC_{0–24}$/MIC ratio of 30) by following a 400-mg once daily adult and a 10-mg/kg/day pediatric gatifloxacin dosing regimen. The *gray* bars represent the distribution of the MICs of gatifloxacin for *B. anthracis*

Figure 13.16 (below) demonstrates that both fluoroquinolones were significantly protective.

The data set provided to the FDA, consisting of all the in vitro data plus the animal model data, resulted in granting of an indication for levofloxacin for postexposure prophylaxis therapy for *B. anthracis*.

Another fluoroquinolone (gatifloxacin) was also examined in a murine inhalation challenge model (Ambrose et al. 2007). Ambrose et al. performed the study with a hyperfractionation (humanization) of the drug administration on a daily, twice, and thrice daily schedule. Examination of Fig. 13.17a demonstrates that $AUC_{0–24}$/MIC ratio was most closely linked to survivorship. Of importance, relatively low AUC/MIC ratios (circa 20) are associated with a near-maximal likelihood of survival.

The authors also used a very conservative free drug target of an AUC/MIC of 30 and looked at the target attainment probability as a function of MIC. In Fig. 13.17b, both children and adults have a target attainment in excess of 95 % for an MIC value of 0.5 mg/L, which is the highest value observed in a collection of 30 strains of *Bacillus anthracis*.

This demonstrates that, as was the case with levofloxacin, the fluoroquinolone gatifloxacin provides therapy which is highly likely to be protective in the postexposure prophylaxis mode for anthrax.

Yersinia pestis

Like *Bacillus anthracis*, *Yersinia pestis* is an agent of possible bioterror. *Y. pestis* is the causative pathogen of plague. Historically, streptomycin has been accorded drug-of-choice status for infections with plague. Again, quinolones have been evaluated as therapeutic agents for this pathogen and may now be the drugs of choice. Louie et al. (2007) compared streptomycin at 1 g Q12h to 500 mg daily of levofloxacin simulated in a hollow fiber infection model. In Fig. 13.18 (below), it is clear that both drugs produce an excellent initial fall in CFU/mL. Streptomycin displays rapid resistance emergence, whereas levofloxacin maintains the cell kill without resistance emergence and finally sterilizes the system.

As part of the evaluation, resistant mutants selected on streptomycin- and levofloxacin-containing agar plates were tested in the mouse thigh model, both with and without granulocytes. Two of three levofloxacin-resistant isolates were poorly biofit and were cleared from the infection site in the absence of drug therapy. Neither streptomycin-resistant isolate was poorly biofit. In the neutropenic setting, there was >2 Log_{10} (CFU/g) outgrowth over time for the wild-type isolate as well as one levofloxacin-resistant isolate and two streptomycin-resistant isolates. For two of the three levofloxacin-resistant isolates, there was a net 2.5 Log_{10} (CFU/g) decline. In the neutrophil replete model, there was a 0.5–0.75 Log_{10} (CFU/g) decline for the wild-type isolate, for both streptomycin-resistant isolates and for one of the levofloxacin-resistant isolates. The other two levofloxacin-resistant isolates had a 5 Log_{10} (CFU/g) drop mediated by the granulocytes over 7 days (data not shown).

Because naturally occurring resistance to both streptomycin and doxycycline has been described, Louie et al. (2011a) examined a fluoroquinolone (moxifloxacin) to identify a dose and schedule that would both kill the pathogen and prevent amplification of resistant mutant subpopulations. In the first experiment, dose ranging studies were performed with the hollow fiber infection model. All simulated doses were administered once daily. Resistant mutants were sought by identifying growth on agar plates containing 2.5× baseline MIC for moxifloxacin. In this way, a 24-h exposure could be identified that would both be permissive (the lower exposure) and nonpermissive (higher exposure) for resistance emergence (see Fig. 13.19, below).

By inspection, exposures equivalent to 200 mg per day of moxifloxacin provided rapid sterilization for the system in 1–3 days. Lower exposures allowed amplification of resistant mutants. Another experiment was performed to identify whether Peak/MIC ratio, AUC/MIC ratio, or Time > MIC was the pharmacodynamic index most closely linked to resistance suppression for moxifloxacin.

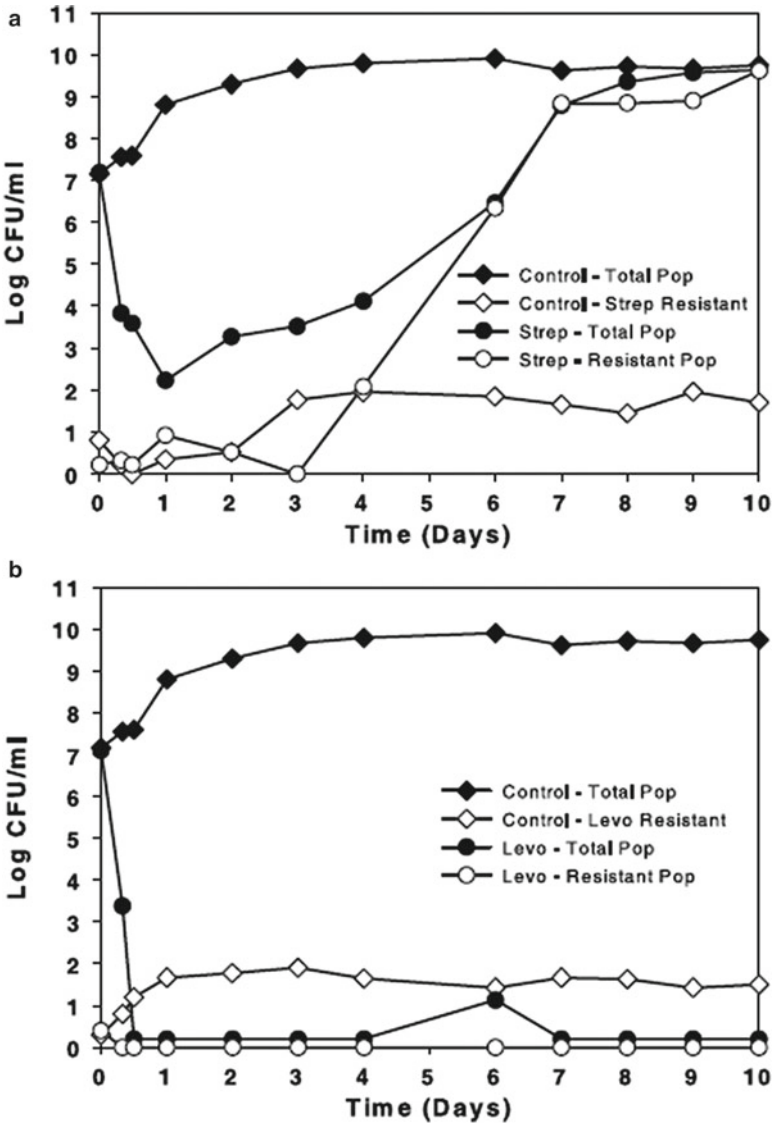

Fig. 13.18 Effects of streptomycin and levofloxacin therapies on the total *Y. pestis* population and the mutant populations with $\geq 3 \times$MICs to (**a**) streptomycin and (**b**) levofloxacin. Streptomycin was given to simulate the human serum concentration–time profile for 1 g i.v. every 12 h, and levofloxacin dosing simulated the human serum concentration–time profile for levofloxacin given orally at 500 mg every 24 h. The studies with streptomycin and levofloxacin therapy were conducted simultaneously

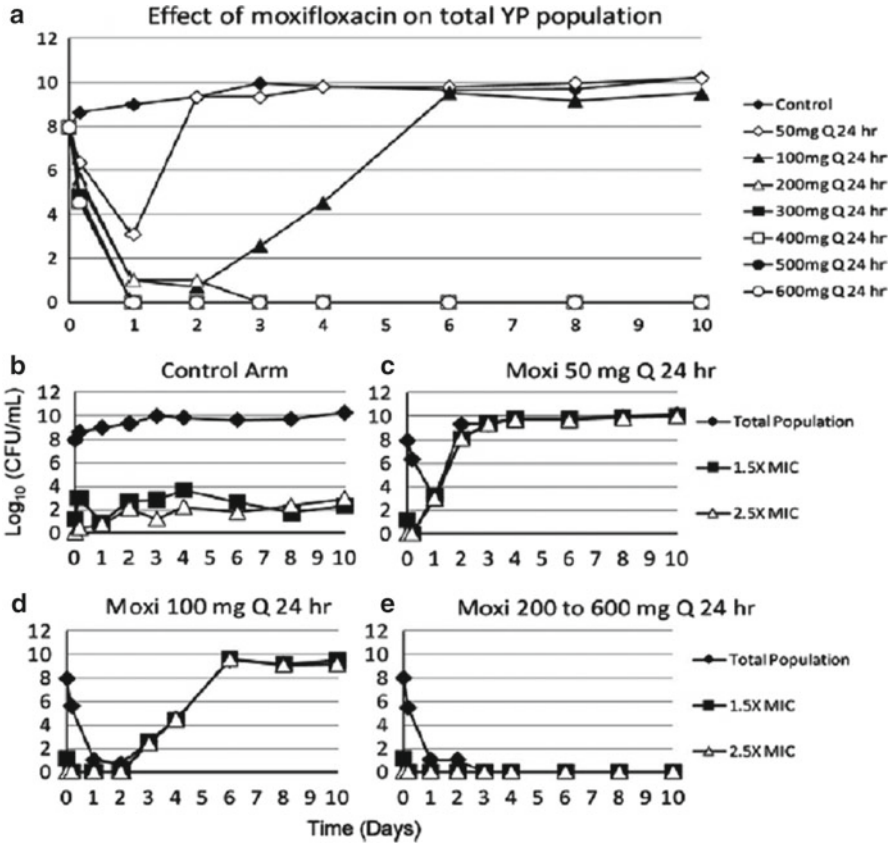

Fig. 13.19 Dose range study of moxifloxacin against *Y. pestis* **Δ**CO92. (**a**) Microbiological effect of each regimen on the total bacterial population. (**b–e**) Effects of individual drug regimens on the total population and the populations that were resistant to 1.5× and 2.5× the MIC values for the parent strain

The experiment was performed in two stages, with a first experiment identifying the important exposure range, with two modes of drug administration, once daily and by continuous infusion. After the important exposure range was identified, a finer mesh of exposures was investigated. This allowed a very well-defined exposure to be identified that would give excellent cell kill as well as suppress resistance. This can be seen in Fig. 13.20.

The critical range for resistance suppression evaluation is 100–200-mg equivalent daily dosing (from Panel a). In Panel (b), the results are clear-cut. At 100-mg equivalent, both daily dosing and continuous infusions fail. At all other exposures, continuous infusions fail in a time-dependent manner with resistance occurring later with higher exposures. No resistance was seen in any daily dosing regimen,

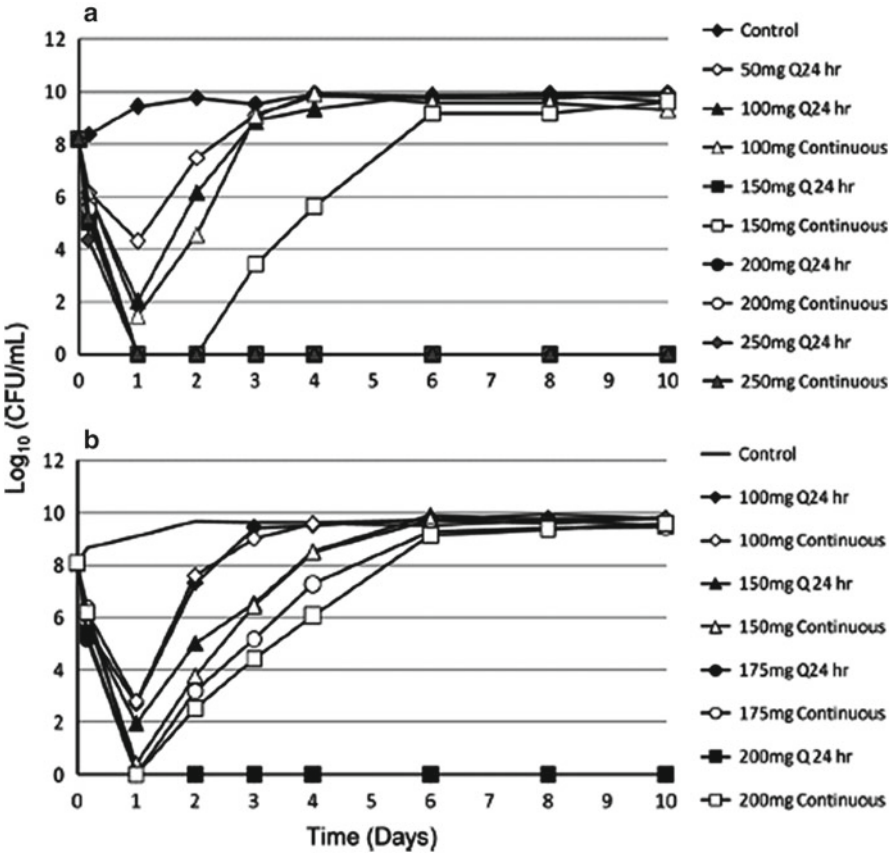

Fig. 13.20 Two dose fractionation studies for moxifloxacin against *Y. pestis* **Δ**CO92. The effect of each regimen on the total bacterial population is shown. The regimens selected for fractionation in the second experiment (**b**) were selected based on the results of the first study (**a**)

identifying Peak/MIC ratio as the pharmacodynamic index most closely linked to resistance suppression.

It should be noted that large populations of patients will have a distribution of clearance values. A Monte Carlo simulation was performed for moxifloxacin to identify the portion of the population that would achieve the exposure target, given once daily, that would suppress amplification of resistant mutant subpopulations. H.S. Heine provided a 30 isolate *Y. pestis* distribution of moxifloxacin MIC values. When the expectation was taken, a 400 mg daily moxifloxacin dose (FDA-approved dose and schedule) would suppress resistance with a 99.7 % probability.

Louie et al. (2011b) also examined fluoroquinolones against five other antimicrobials in the hollow fiber infection model. Again, quinolones, at standard doses and schedules, provided the most robust cell kill and resistance suppression (data not shown).

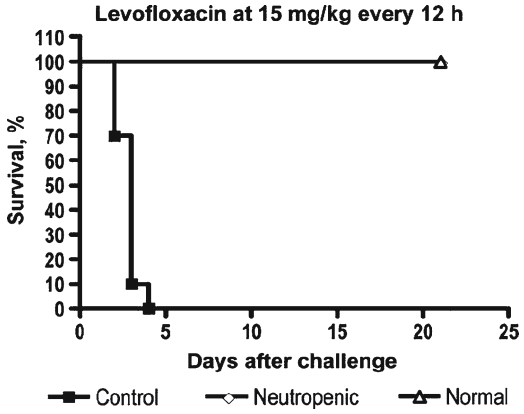

Fig. 13.21 Evaluation of the impact of neutropenia induced by cyclophosphamide on the survivorship of BALB/c mice challenged with *Yersinia pestis* CO92 by aerosol administration and treated with levofloxacin. The results demonstrate that survivorship is not affected by granulocytopenia

It is tempting to give the "drug-of-choice" title to the quinolones on the basis of the hollow fiber model data. However, the FDA requires animal model validation. Heine et al. (2007b) examined levofloxacin in a murine inhalational challenge model. The results are displayed in Fig. 13.21.

As seen in the in vivo system, the fluoroquinolone performed excellently, with a 100 % survivorship of infected mice. This was true whether or not the mice were rendered granulocytopenic or were neutrophil replete. Currently, work is ongoing in the nonhuman primate (NHP) for both moxifloxacin and levofloxacin to complete the data set for the FDA. Should these drugs work as well as expected in the NHP, it would be a reasoned judgment to confer the "drug-of-choice" appellation on them.

Conclusion

Whether one looks at hospital-acquired or community-acquired infection, the therapy of *Mycobacterium tuberculosis* or the therapy of select agents, the fluoroquinolones have shown themselves to be an important part of the clinician's armamentarium. Uniquely, the fluoroquinolones development has coincided with the rise of pharmacodynamic analysis both preclinically (pharmacodynamic in vitro as well as animal model systems) and at the bedside. New mathematical tools (stochastic optimal design, population modeling, Bayesian estimation) linked to appropriate statistical analysis has brought this about. In this era of multiresistance, dose and schedule optimization for cell kill and resistance suppression will help keep the quinolones an important part of the clinician's armamentarium.

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Chapter 14
Pharmacokinetics and Pharmacodynamics of Colistin

Roger L. Nation, Phillip J. Bergen, and Jian Li

Abstract The increasing prevalence of multidrug-resistant Gram-negative bacteria worldwide has resulted in colistin, administered as its inactive prodrug colistin methanesulfonate (CMS), being increasingly used as a last-line therapy to treat infections caused by these pathogens. Developed well before contemporary drug development procedures, substantial improvements in the understanding of its chemistry, pharmacokinetics (PK), pharmacodynamics (PD) and PK/PD relationships have occurred over the last decade which have enabled substantial progress towards optimising its clinical use in different patient populations. This has resulted in the first scientifically based dosing algorithm for various categories of critically ill patients receiving CMS to generate a desired target steady-state plasma concentration of formed colistin. It has become clear that monotherapy with CMS is unlikely to generate plasma colistin concentrations that are reliably efficacious. With nephrotoxicity preventing simply increasing the dose of CMS, combination therapy may be required in order to maximise efficacy and minimise the emergence of resistance.

Keywords Colistin • Colistin methanesulfonate • Pharmacokinetics • Pharmacodynamics • Dose optimization • *P. aeruginosa* • *A. baumannii* • *K. pneumoniae* • Multidrug resistance

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Introduction

The increasing prevalence of infections caused by multidrug-resistant (MDR) Gram-negative bacteria, especially *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, and the dearth of new antibiotics with activity against these pathogens reaching the clinic (Livermore 2004; Boucher et al. 2009; Opal and Calandra 2009; IDSA 2010; Spellberg et al. 2011), mean that physicians are needing to resort to use of the polymyxin class of antibiotics (Li et al. 2006b; Landman et al. 2008; Nation and Li 2009; Lim et al. 2010; Michalopoulos and Karatza 2010). These are 'old' antibiotics that were discovered in the late 1940s and then became available in the clinic about a decade later. While a number of polymyxins are known, only two (polymyxin B and E, the latter also known more commonly as colistin) are used clinically. Of the two polymyxins, colistin is most commonly used in the majority of places in the world and is the subject of this chapter.

Drug development and regulatory approval processes were much different in the 1950s than those existing today. As a result, colistin was never subjected to the scientific rigour required of modern pharmaceuticals before they become available for use in patients. It is not surprising, therefore, that there has been a major paucity of pharmacological and other scientific information that is needed to guide rational use of colistin in various categories of patients. Over the last decade or so, since the resurgence in its clinical use, there have been a small number of research groups conducting studies to establish the scientific basis for clinical utilisation of colistin. In essence this antibiotic, that is more than half a century old, has been subjected over the last few years to scientific investigation and evaluation that is akin to the drug development procedures required of newly discovered pharmaceuticals.

At the outset, it is important to indicate what will, and what will not, be reviewed in this chapter. The literature supporting, or otherwise, the clinical efficacy of colistin will not be examined. Not unexpectedly in view of the history surrounding colistin, there is a shortage of information relating to clinical efficacy. Most reports have been based upon retrospective studies and randomised controlled trials are conspicuous by their absence; those interested may consult other sources of information (Li et al. 2006b; Landman et al. 2008; Molina et al. 2009). Like all other drugs, use of colistin may be associated with adverse effects. The most worrying potential adverse effect of colistin is nephrotoxicity (Falagas and Kasiakou 2006; Hartzell et al. 2009; DeRyke et al. 2010; Kwon et al. 2010; Ko et al. 2011). Fortunately, if this adverse effect occurs, it is usually mild to moderate in nature and reversible upon discontinuation of colistin therapy (Falagas et al. 2005; Falagas and Kasiakou 2006; Betrosian et al. 2008; Pintado et al. 2008; Hartzell et al. 2009). Colistin-induced nephrotoxicity appears to involve accumulation of colistin in renal tubular cells mediated by transporters (Li et al. 2003c, 2004; Ma et al. 2009) and ensuing oxidative stress (Ozyilmaz et al. 2011; Yousef et al. 2011, 2012). Clinically used antioxidants, *N*-acetylcysteine, melatonin and ascorbic acid, have been shown in animal models to ameliorate colistin-induced nephrotoxicity (Ozyilmaz et al. 2011; Yousef et al. 2011, 2012) and they hold promise for application in patients.

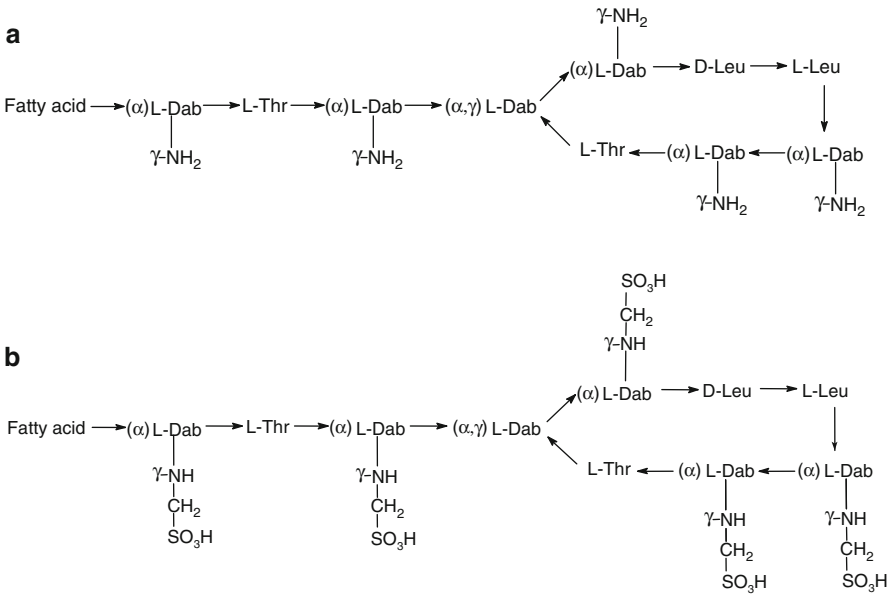

Fig. 14.1 Structures of (**a**) colistin A and B and (**b**) colistin A and B methanesulfonate. Fatty acid: 6-methyloctanoic acid for colistin A and 6-methylheptanoic acid for colistin B; *Thr* threonine, *Leu* leucine, *Dab* α,γ-diaminobutyric acid. α and γ indicate the respective amino group involved in the peptide linkage

Nephrotoxicity will not be further reviewed here. This chapter will provide an overview of the current state of microbiological and pharmacological knowledge, especially in relation to the pharmacokinetic (PK) and pharmacodynamic (PD) properties of colistin, and the PK/PD driver of its antibacterial effect. Because an appreciation of the chemistry and related terminology (including that applied to the material used in the clinic) is essential to an understanding of the pharmacological and microbiological properties of colistin, there is no option but to briefly review these aspects.

Important Aspects of Chemistry

Colistin is a polypeptide antibiotic produced by *Bacillus colistinus* (Koyama et al. 1950). It comprises a heptapeptide ring with a tripeptide side chain to which is covalently linked a fatty acyl tail. It is a multicomponent antibiotic, with colistin A (polymyxin E1) and colistin B (polymyxin E2), which differ by only one carbon and two protons in the fatty acyl tail, being the two major components (Koyama et al. 1950; Orwa et al. 2000, 2001) (Fig. 14.1a). Because of the biological origin of this antibiotic, there is supplier-to-supplier and batch-to-batch variation in the proportion

of colistin A and colistin B in commercial material (Decolin et al. 1997; Li et al. 2001a). At physiological pH, the primary amines in the α,γ-diaminobutyric acid (Dab) residues (p*K*a approximately 10) are ionised; thus, colistin is a cationic antimicrobial peptide. Because colistin contains both polar (by virtue of the ionised Dab residues) and hydrophobic regions (fatty acyl tail), the molecule is amphiphilic and thereby exhibits surface activity (Wallace et al. 2010). As discussed below, both the cationic Dab residues and the hydrophobic fatty acyl tail are important for the interaction of colistin with the outer membrane of Gram-negative bacteria, a key first step in the bactericidal action of this antibiotic. Colistin is available commercially in the form of its colistin methanesulfonate salt. Colistin methanesulfonate is not administered parenterally, but is used in some countries in topical pharmaceutical formulations.

Colistin methanesulfonate (CMS, also known as colistimethate; Fig. 14.1b) is the form of 'colistin' that is administered parenterally and by inhalation. The sodium salt of CMS, in lyophilized form, is present in parenteral (and inhalational) formulations. It is crucial to understand the relationship between CMS and colistin. CMS was developed during the 1950s because of concerns in early studies about the relatively high level of toxicity that was associated with parenteral administration of colistin methanesulfonate. CMS is prepared from colistin by reaction of the free γ-amino groups of the five Dab residues with formaldehyde followed by sodium bisulfite (Barnett et al. 1964; Beveridge and Martin 1967). CMS is an inactive prodrug (Bergen et al. 2006) and is converted in vivo to the active antibacterial entity, colistin (Li et al. 2003a, 2004, 2005b; Markou et al. 2008; Plachouras et al. 2009; Imberti et al. 2010; Marchand et al. 2010a; Couet et al. 2011; Garonzik et al. 2011; Mohamed et al. 2012). It is essential to understand that the conversion of CMS to colistin, a requirement for antibacterial activity in vivo, may also occur in vitro as CMS is not stable in aqueous environments (Li et al. 2003b; Wallace et al. 2008b, 2010). Thus, the conversion of CMS to colistin, via a number of partially sulfomethylated derivatives, has been demonstrated to occur not only in vivo (see below) but also in vitro in plasma, urine, buffer solutions and microbiological culture medium (Li et al. 2003b, 2004; Bergen et al. 2006). The conversion of CMS to colistin also occurs in the solutions for administration to patients that are produced by reconstitution of the lyophilized powder in pharmaceutical products (Wallace et al. 2008b); to minimise this in vitro conversion, such reconstitution should occur immediately prior to administration. An awareness that CMS converts to colistin in aqueous media highlights the importance in PK and PK/PD studies of ensuring that blood, plasma and other biological samples are processed and stored appropriately to avoid in vitro conversion occurring after collection of samples (Dudhani et al. 2010a). Such conversion would result in a spuriously high estimate of the colistin concentration present in the sample at the time of its collection. Moreover, an appreciation of the facile conversion of CMS to colistin in aqueous media clearly leads to the conclusion that colistin methanesulfonate, rather than CMS sodium, must be used in determination of minimum inhibitory concentration (MIC). The use of CMS in MIC determinations will lead to an 'apparent' MIC that will represent the activity of the progressively increasing amount of colistin liberated from the CMS during the

course of the microbiological incubation (Bergen et al. 2006). The time course of liberation of colistin may vary from laboratory to laboratory dependent upon conditions employed, and this would be expected to lead to variability in MIC values for a given strain.

Antibacterial Properties

Spectrum of Activity

Colistin exhibits a narrow antibacterial spectrum of activity, mostly against common Gram-negative pathogens (Li et al. 2005a). Colistin retains excellent in vitro bactericidal activity against most common species of Gram-negative bacilli or coccobacilli including *P. aeruginosa* (Walkty et al. 2009; Cernohorska and Slavikova 2010; Gales et al. 2011), *Acinetobacter* spp. (Walkty et al. 2009; Yau et al. 2009; Gales et al. 2011; Queenan et al. 2012) and *Klebsiella* spp. (Walkty et al. 2009; Hawser 2010; Gales et al. 2011; Sader et al. 2011), the organisms against which it is most commonly used clinically. Activity against other Gram-negative bacterial species has been reviewed elsewhere (Falagas and Kasiakou 2005; Li et al. 2005a). Colistin has no significant activity against most Gram-positive bacteria (Schwartz et al. 1959; Finland et al. 1976a, b) or fungi (Hoeprich 1970).

Susceptibility Breakpoints

The breakpoints for colistin susceptibility are based on colistin methanesulfonate given that CMS is an inactive prodrug (Bergen et al. 2006), and different breakpoints have been employed by various organisations (Comite de l' Antibiogramme de la Societe Francaise de Microbiologie (SFM) 2005; Andrews and Howe 2011; Clinical and Laboratory Standards Institute (CLSI) 2012; European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2012). The Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoints are $\leq$2 mg/L for both *P. aeruginosa* and *A. baumannii* using the microbroth dilution method (Clinical and Laboratory Standards Institute (CLSI) 2012). Given the emerging clinical pharmacokinetic and pharmacodynamic data (see sections below), the appropriateness of these breakpoints within a clinical context remains to be determined. Worryingly, colistin heteroresistance, the presence of resistant subpopulations within an isolate that is susceptible based upon its MIC, has been observed in *A. baumannii* (Li et al. 2006c; Owen et al. 2007; Tan et al. 2007; Hawley et al. 2008; Yau et al. 2009), *K. pneumoniae* (Poudyal et al. 2008; Meletis et al. 2011) and *P. aeruginosa* (Bergen et al. 2011a). While colistin still retains excellent activity generally, resistance to

colistin is increasing in several key species including *P. aeruginosa* (Johansen et al. 2008; Lee et al. 2011), *A. baumannii* (Ko et al. 2007; Al-Sweih et al. 2011), *K. pneumoniae* and other *Enterobacteriaceae* (Tan and Ng 2006; Kontopoulou et al. 2010; Suh et al. 2010; Toth et al. 2010; Bogdanovich et al. 2011; Marchaim et al. 2011; Mezzatesta et al. 2011), and *S. maltophilia* (Tan and Ng 2006).

Mechanisms of Activity and Resistance

When considering the mechanism of antimicrobial activity it must be remembered that the polymyxins are polycationic, amphiphilic peptides. As colistin and polymyxin B are structurally similar, differing by only one amino acid, they are believed to share the same mechanism of antibacterial action. The initial target of the polymyxins against Gram-negative bacteria is the lipopolysaccharide (LPS) component of the outer membrane, initiated by electrostatic attraction between the cationic polymyxin molecule and the anionic lipid A of LPS, thereby displacing divalent inorganic cations (Ca^{2+}, Mg^{2+}) that assist in stabilising the LPS leaflet (Hancock and Chapple 1999). Once electrostatically bound to LPS, the *N*-terminal fatty-acyl tail is inserted into the outer membrane in a process driven by hydrophobic interactions. The overall result is permeabilization of the outer membrane, allowing the polymyxin to access the periplasmic space and the cytoplasmic membrane; this is the so-called 'self-promoted uptake' mechanism (Hancock and Chapple 1999). Originally it was proposed that the polymyxin inserted into the cytoplasmic membrane forming conductance events leading to leakage of cell contents and cell death (Hancock et al. 1995). However, there is an increasing body of evidence that suggests the polymyxins exert their effects through an alternative mode of action or that they may in fact act upon multiple bacterial cell targets (Hancock and Rozek 2002; Brogden 2005; Hale and Hancock 2007). The exact mechanism(s) by which they ultimately kill bacterial cells is still unknown.

Given that the crucial first step in the action of polymyxins on Gram-negative bacterial cells is the electrostatic interaction between the positively charged polymyxins and the negatively charged LPS, it is not surprising that resistance to polymyxins often involves changes in LPS structure which decrease the negative charge on the cell surface and hence the electrostatic interactions with the peptide. Modifications to the lipid A and/or core of LPS typically mask phosphate groups with moieties such as aminoarabinose and phosphoethanolamine. Such modifications have been observed in *P. aeruginosa* (Moskowitz et al. 2004), *K. pneumoniae* (Helander et al. 1996) and other bacterial species (Morrison and Wenzel 1984; Breazeale et al. 2005; Winfield et al. 2005) and have been shown to increase resistance to polymyxins (Moskowitz et al. 2004; Breazeale et al. 2005; Lewis et al. 2009; Beceiro et al. 2011). Interestingly, it was recently shown that resistance in *A. baumannii* can be mediated by complete loss of LPS (Moffatt et al. 2010). In *K. pneumoniae*, the presence of capsule may also be important for polymyxin resistance (Helander et al. 1996; Llobet et al. 2008).

Inconsistent Labelling and Dose Regimens of Pharmaceutical Products

Unfortunately, different conventions are used in various parts of the world for labelling the content of CMS pharmaceutical products and in defining the recommended daily doses (Li et al. 2006a, b). In some parts of the world (e.g. Europe), CMS parenteral vials are labelled in international units (IU). In these countries there are usually three vial sizes containing 500,000 IU, 1 million IU and 2 million IU per vial, corresponding to approximately 40, 80 and 160 mg of CMS sodium per vial (since there are ~12,500 IU per mg of CMS sodium). In many countries in the world (e.g. USA, Canada, Australia), the parenteral product available is labelled in terms of 'colistin base activity'. In these countries, one vial size only is available and this contains 150 mg of colistin base activity, which actually corresponds to ~400 mg CMS sodium.

Very unfortunately, the inconsistency does not end with labelling of the pharmaceutical products; it extends to the recommended daily doses in the respective product information. For those products labelled in international units, the typical recommended dose for a patient over 60 kg and with normal renal function is 1–2 million IU three times daily (Li et al. 2006b), equivalent to 240–480 mg CMS sodium per day. For those products labelled in terms of colistin base activity, the recommended doses are 2.5–5 mg/kg colistin base activity per day in 2–4 divided doses (Li et al. 2006b), which is equivalent to about 6.67–13.3 mg/kg of CMS sodium per day. Thus, for a patient with normal renal function and bodyweight of 60 kg, the recommended dose of such a product labelled in terms of colistin base activity (recommended dose of 400–800 mg CMS sodium per day) is almost double that of the products that are labelled in international units (recommended dose of 240–480 mg CMS sodium per day, see above). The origin of this major discrepancy in recommended doses between products appears to be lost in the sands of time. Because both regimens appear to be equally well tolerated and because of the need to ensure the maximum antibacterial effect in an era of increasing multidrug resistance and shortage of new antibiotics, the higher of the two recommended dosage regimens (i.e. 2.5–5 mg/kg colistin base activity per day, equivalent to 400–800 mg CMS sodium per day) would seem to be a wise choice; as discussed below, a reduction in the daily dose may be appropriate for patients with impaired renal function.

The inconsistent labelling convention and the discrepant recommended dosage regimens have the potential to cause much confusion; indeed, even those clinicians who are familiar with the use of CMS are often unaware of this problem. Tragically, the confusion surrounding CMS labelling recently resulted in the death of a patient in the USA (Institute for Safe Medication Practices (ISMP) 2011). In that case, the physician ordered the dose as mg of CMS rather than as colistin base activity, the usual method of expressing the dose in the USA. This went unrecognised by the pharmacist and nurses and resulted in the patient receiving doses ~2.7-fold higher than intended. The patient subsequently developed acute renal failure and other complications that resulted in their death. Calls a number of years ago for an

international consensus (Li et al. 2006a) have not been actioned. Clinicians reading the international literature to inform their practice will need to remain vigilant in regard to interpretation of dosage regimens used in published studies.

Pharmacokinetics, Pharmacodynamics and Pharmacokinetic/Pharmacodynamic Relationships

Important Methodological Considerations for PK, PD and PK/PD Studies

At the outset, it is essential to comment upon the analytical methods that have been employed in PK studies over the years. Such studies conducted through the 1950s to the 1990s inclusive were undertaken using microbiological assays (Mackay and Kaye 1964; al-Khayyat and Aronson 1973); indeed, such assays have been used in some of the more recent studies (Aoki et al. 2009). These assays, when applied to biological samples containing both CMS and colistin formed in vivo, are not capable of differentiating between the colistin actually present in the sample at the time of its collection from a subject administered CMS and the colistin formed in vitro during the incubation period of the microbiological assay. Thus, the use of such assays is incapable of providing accurate information on the time course of plasma concentrations of the prodrug (CMS) and the active entity (colistin). The pharmacokinetic characteristics described within the current prescribing information for the various parenteral products was obtained using microbiological assays; thus it is unhelpful and does not provide a solid scientific basis for understanding the disposition of administered CMS and the colistin formed from it in vivo.

An accurate and complete understanding of the PK of CMS and formed colistin has only been possible over the last 5–10 years since the development of HPLC (Li et al. 2001a, 2002) and LC/MS/MS (Jansson et al. 2009; Gobin et al. 2010; Dotsikas et al. 2011) analytical methods for the separate quantification of CMS and colistin in biological samples. Here, a couple of important points should be made. Firstly, all of the current methods for HPLC or LC/MS/MS analysis of 'CMS' involve so-called 'difference assays'. That is, the 'CMS' concentration in a biological sample is determined as the difference between the colistin concentration measured in a sample that has been carefully processed and stored to prevent in vitro conversion of CMS to colistin and the colistin concentration in another aliquot of the sample where the conversion of CMS to colistin is forced to occur in vitro. The 'CMS' concentration determined using this approach represents the concentration of CMS (i.e. the penta-sulfomethylated species) and the numerous partially sulfomethylated species that are intermediates in the conversion of CMS to colistin. This type of analytical approach has been necessary because it has not been possible to directly quantify CMS due to the complex chemical nature and composition of CMS. Secondly, it is essential to appreciate that very careful procedures must be employed

in the handling and storage of biological samples to avoid the in vitro conversion of CMS to colistin. Such in vitro conversion would lead to an underestimation of the 'CMS' concentration and, more importantly, an overestimation of the colistin concentration in the biological sample. Thus, upon their collection, samples must be placed on ice, processed rapidly (e.g. blood samples centrifuged to obtain plasma/serum) and stored under conditions to minimise in vitro conversion of CMS to colistin prior to analysis. In regard to the latter, it has been demonstrated that storage of plasma samples at −20 °C is generally not acceptable, unless the samples are analysed within 1 month of collection (Dudhani et al. 2010a). Samples should be stored at −70 °C to −80 °C and even then the samples must be analysed within 4 months of collection to avoid substantial conversion of CMS into colistin and the degradation of both entities.

Overview of the Pharmacokinetics of CMS and Formed Colistin

The availability in the past decade or so of liquid chromatographic methods has enabled increased understanding of the relatively complex disposition of the inactive prodrug, CMS and the (active) colistin formed from it in the body. The intravenous route is the most common way in which CMS is administered, especially in critically ill patients with life-threatening infections caused by Gram-negative bacteria. For this reason, and also because PK data obtained from studies using this route are the most informative in regard to dispositional characteristics, the major focus here will be on studies conducted following intravenous administration. This section will provide an overview of preclinical PK studies; this is important because there are aspects of the overall PK of CMS and formed colistin that are only possible to reveal by undertaking studies involving separate administration of CMS and preformed colistin, which cannot be readily performed in humans.

The differences in chemistry between CMS and colistin (see section entitled 'Important Aspects of Chemistry') translate into differences in the PK of these entities. Li et al. was the first to apply HPLC methods capable of distinguishing between CMS and colistin to studies undertaken in rats administered either CMS (Li et al. 2004) or colistin (Li et al. 2003c). Those studies provided very useful information concerning the differences in disposition of CMS and the colistin formed from it in vivo. Following intravenous administration of CMS in rats, colistin appeared in plasma soon after administration of the prodrug (Li et al. 2004). The terminal half-life of formed colistin was approximately twice that of the administered CMS and was similar to the half-life of colistin administered directly (Li et al. 2003c). This indicated that the overall disposition of formed colistin following administration of CMS was rate limited by its elimination rather than its formation. The fundamental aspects of the overall disposition of CMS and formed colistin observed by Li et al. (2004) were subsequently confirmed by Marchand et al. using a wide range of CMS doses (5–120 mg/kg intravenously) in a rat PK study (Marchand et al. 2010b). In people with cystic fibrosis, the terminal half-life of formed colistin (251 ± 79 min;

mean ± SD) has also been reported to be approximately twice that of the administered CMS (124 ± 52 min) (Li et al. 2003a). More recent studies conducted in critically ill patients indicated that the terminal half-life of formed colistin is substantially longer (up to ~18 h) than that of the CMS (~3 h) that was administered (Markou et al. 2008; Plachouras et al. 2009; Garonzik et al. 2011; Mohamed et al. 2012); it is also evident that the half-life of formed colistin in critically ill patients is longer than that in people with cystic fibrosis (Li et al. 2003a), which may relate to differences in renal function and other patient characteristics (see section 'How Appropriate Are Current Dosage Regimens?'). From studies conducted to date, the PK of CMS and formed colistin appears to be linear following intravenous administration of CMS. In rats administered intravenous CMS across the range 5–120 mg/kg (which generated plasma concentrations of CMS and formed colistin that span those that are clinically relevant) linear relationships were observed between CMS and colistin areas under the plasma concentration–time curves (AUC) to infinity and CMS doses, as well as between CMS and colistin maximum plasma concentration (C_{max}) values and CMS doses (Marchand et al. 2010b). Following direct administration of colistin subcutaneously across a range of colistin doses to infected neutropenic mice, there was evidence of non-linear PK (plasma colistin concentration increased to a greater extent than the increase in dose) (Dudhani et al. 2010b); however, this may have resulted from non-linearity in the tissue binding of colistin, including at the subcutaneous site of administration, thereby impacting the fraction of the dose available for absorption.

Studies performed several decades ago employing microbiological assays indicated that colistin binds extensively to tissues of many organs, whereas a lesser degree of tissue binding was apparent for CMS (Kunin and Bugg 1971; Craig and Kunin 1973; Ziv et al. 1982; Leroy et al. 1989). The studies with CMS must be interpreted cautiously due to the use of a microbiological assay, which is non-specific for CMS as the assay measures the concentration of active colistin generated from CMS in vivo as well as during the incubation period of the microbiological assay. Protein binding studies in plasma from a range of healthy (i.e. non-infected) animals indicated that colistin was 30–70 % plasma bound (Ziv and Sulman 1972; al-Khayyat and Aronson 1973; Li et al. 2003c). A recent study of colistin binding in plasma from infected neutropenic mice indicated that the binding of colistin was higher than that above (Dudhani et al. 2010b). It is apparent that colistin binds to both albumin and α_1-acid glycoprotein (Dudhani et al. 2009), the latter being an acute-phase reactant protein whose plasma concentration increases in a variety of stressful conditions, including infection (Voulgari et al. 1982; Morita and Yamaji 1995). The extent of plasma binding of colistin in infected patients may therefore be subject to variations in the concentrations of albumin, α_1-acid glycoprotein and any other proteins involved in its binding.

There are very substantial differences in the clearance pathways for CMS and colistin. Following intravenous administration of colistin in rats, substantially less than 1 % of the dose was recovered in urine in unchanged form (Li et al. 2003c); the renal clearance involved very extensive renal tubular reabsorption to an extent greater than that occurring for water indicating that the reabsorption of colistin must

be a carrier-mediated process (Li et al. 2003c; Ma et al. 2009). The very minor role for renal clearance in the overall body clearance of colistin was also observed for polymyxin B (differing from colistin in just one amino acid) in patients (Zavascki et al. 2008). In marked contrast, CMS was shown to be predominantly renally cleared in rats with a component of tubular secretion (Li et al. 2004). The trafficking through renal tubular cells of CMS by secretion [with the possibility of intracellular generation of colistin (Li et al. 2004)] and of colistin by tubular reabsorption (Li et al. 2003c) may explain in part the propensity for nephrotoxicity following administration of CMS. Comparison of the dose-normalised AUC of formed colistin arising from administration of CMS in rats with that arising from direct administration of colistin allowed estimation of the fraction of the dose of CMS that was converted systemically to colistin (Li et al. 2004); this revealed that only a very small proportion (~7 %) of the administered dose of CMS was converted to colistin. A subsequent study in rats by Marchand et al. (Marchand et al. 2010b) confirmed many of the observations of Li et al. (Li et al. 2004). Similar to the findings in rats, Couet et al. recently demonstrated that in young healthy volunteers administered a single dose of one million IU of CMS (infused over 1 h), CMS was predominantly excreted in the urine (70 % on average as both CMS and colistin, the majority of the latter forming in the urinary tract) (Couet et al. 2011). The low in vivo fractional conversion of the prodrug, CMS, to the active form, colistin, arises because the conversion clearance of CMS to colistin is substantially lower than the renal clearance of CMS (i.e. the fractional conversion is dictated by the relative efficiencies of parallel pathways for elimination of CMS).

As a result of the understanding generated from these studies, the overall disposition of CMS and formed colistin has been summarised as shown in Fig. 14.2. The schema shown in Fig. 14.2 is consistent with the emerging data on the pharmacokinetics of CMS and formed colistin in humans, which is discussed below (see section 'How Appropriate Are Current Dosage Regimens?').

Pharmacodynamics of Colistin

Although colistin is administered parenterally as CMS, it is important to recognise that antimicrobial activity results from formation of colistin, not from CMS or its partially sulfomethylated derivatives (Bergen et al. 2006). Thus, CMS should be considered an inactive prodrug of colistin. Most PD data on colistin have been generated using in vitro models. Time-kill studies with colistin (used as its sulfate salt) in both static and dynamic systems showed potent, concentration-dependent killing against *P. aeruginosa* (Eickhoff and Finland 1965; Li et al. 2001b; Gunderson et al. 2003; Bergen et al. 2008, 2010, 2011a, b; Bulitta et al. 2010; Lin et al. 2010), *A. baumannii* (Owen et al. 2007; Tan et al. 2007) and *K. pneumoniae* (Poudyal et al. 2008; Deris et al. 2012), including multidrug-resistant and colistin-heteroresistant strains. Initial killing is very rapid, with a large decrease in colony-forming units (cfu) per mL occurring as early as 5 min after exposure to colistin concentrations in the vicinity

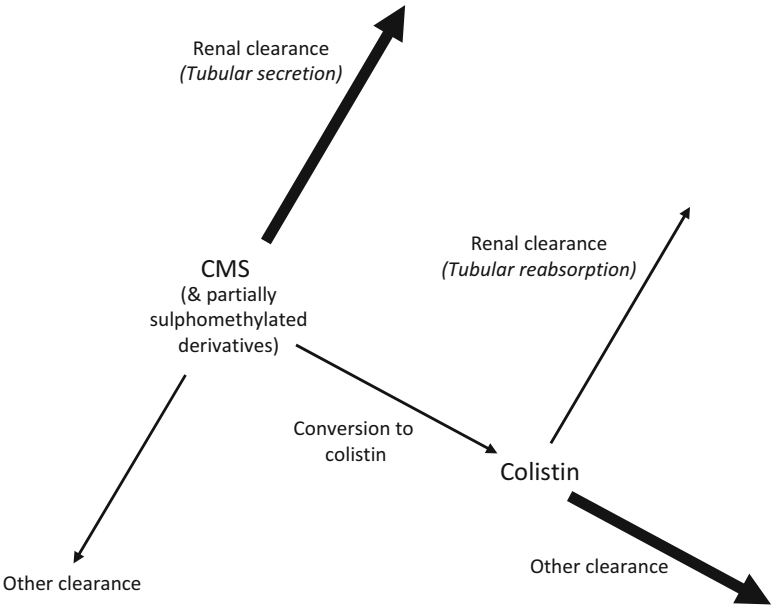

Fig. 14.2 Schematic representation of the disposition of colistin methanesulfonate and the colistin generated from it in the body following administration of sodium colistin methanesulfonate. Modified after (Li et al. 2006b) with permission from Elsevier

of the MIC and above. A modest post-antibiotic effect was found only at high colistin concentrations (Li et al. 2001b; Owen et al. 2007; Poudyal et al. 2008). Both the rate and extent of killing are markedly decreased at high compared to low inocula (Bulitta et al. 2010; Bergen et al. 2011a, b; Deris et al. 2012). Against a genetically characterised isolate of *P. aeruginosa* (PAO1), killing of the susceptible population by colistin was 23-fold slower at an inoculum of 10^9 cfu/mL and sixfold slower at 10^8 cfu/mL compared with 10^6 cfu/mL. Up to 32-fold higher colistin concentrations were required at the 10^9 compared with the 10^6 cfu/mL inoculum to achieve bactericidal activity ($a \geq 3\text{-log}_{10}$ cfu/mL decrease) (Bulitta et al. 2010). Thus, there is a potential need for higher colistin exposure or combination regimens to treat deep-seated, difficult-to-treat infections with high inocula.

A consistent finding of both in vitro and in vivo studies is regrowth with colistin monotherapy, even with concentrations well above those which can be safely achieved clinically. For example, two studies which utilised in vitro PD models (Gunderson et al. 2003; Bergen et al. 2010) reported regrowth of *P. aeruginosa* with colistin concentrations well above clinically achievable levels, the former with concentrations up to 200 mg/L, while similar regrowth has been reported in *A. baumannii* (Owen et al. 2007) and *K. pneumoniae* (Poudyal et al. 2008; Deris et al. 2012) with colistin concentrations up to 64×MIC. Regrowth following colistin monotherapy has been shown to be the result of amplification of colistin-resistant subpopulations (Tan et al. 2007; Bergen et al. 2008, 2011a, b; Poudyal et al. 2008;

Bulitta et al. 2010; Dudhani et al. 2010b; Deris et al. 2012). The difficulty of eradicating colistin-resistant subpopulations with colistin monotherapy, together with the potential for rapid amplification of colistin-resistant subpopulations, suggests caution with the use of colistin monotherapy and highlights the importance of investigating rational colistin combinations.

Which Pharmacokinetic/Pharmacodynamic Index Is Most Predictive of Efficacy?

Only recently have studies used a dose-fractionation design to investigate the relationship between the PK and PD of colistin, namely which PK/PD index [C_{max}/MIC, AUC/MIC, or $T_{>MIC}$ (time for which concentrations exceed the MIC)] best correlates with colistin efficacy (Ketthireddy et al. 2007; Bergen et al. 2010; Dudhani et al. 2010b, c; Hengzhuang et al. 2012). Using an in vitro PK/PD model, Bergen et al. examined 37 different regimens involving various colistin C_{max} and dosage frequencies (including intermittent dosing and continuous infusion regimens) against three strains of *P. aeruginosa* including a colistin-susceptible but MDR strain (Bergen et al. 2010); analysis was based upon unbound or free (f) indices (i.e. fC_{max}/MIC, fAUC/MIC, and $fT_{>MIC}$). The overall killing effect was best correlated with fAUC/MIC ($R^2 = 0.931$); weaker correlations occurred for fC_{max}/MIC ($R^2 = 0.868$) and $fT_{>MIC}$ ($R^2 = 0.785$) (Fig. 14.3). The magnitudes of fAUC/MIC required for 1- and 2-$\log_{10}$ reductions in the area under the cfu/mL curve relative to growth control were able to be determined.

In a conference abstract describing use of a neutropenic mouse thigh infection model, Ketthireddy et al. concluded that once-daily dosing of colistin was most effective against *P. aeruginosa* and that C_{max}/MIC was likely the PK/PD index most predictive of efficacy; PK data, however, were not included in that study (Ketthireddy et al. 2007). Dudhani et al. employed neutropenic mouse thigh and lung infection models in dose-fractionation studies with colistin against three strains each of *P. aeruginosa* and *A. baumannii* which included MDR but colistin-susceptible and, for *A. baumannii*, colistin-heteroresistant strains (Dudhani et al. 2010b, c). In these studies, the time course of total (i.e. protein-bound plus unbound) and unbound plasma colistin concentrations were determined allowing the PK/PD analysis to be based upon unbound indices. The PK/PD index most predictive of the antibacterial effect against both *P. aeruginosa* and *A. baumannii* in both thigh and lung infection models was fAUC/MIC (see Fig. 14.4, for colistin against *P. aeruginosa* in murine lung infection model), in agreement with the results from dose-fractionation studies of colistin against *P. aeruginosa* in an in vitro PK/PD model (Bergen et al. 2010). That fAUC/MIC is the most predictive PK/PD index indicates that time-averaged exposure to colistin is more important than the achievement of high peak concentrations from the administration of larger, less frequent doses. For both *P. aeruginosa* and *A. baumannii*, fAUC/MIC targets required to achieve various magnitudes of

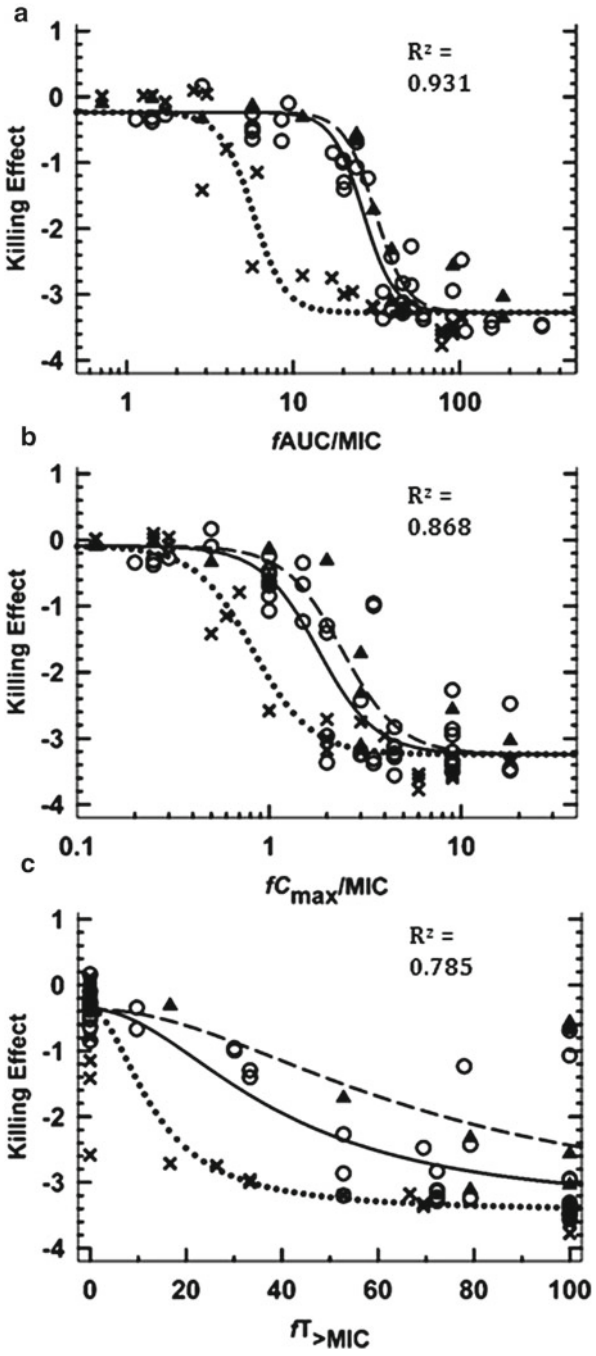

Fig. 14.3 Relationship between killing effect against three strains of *P. aeruginosa* as a function of three PK/PD indices: (**a**) *f*AUC/MIC, (**b**) fC_{max}/MIC and (**c**) $fT_{>MIC}$. ATCC 27853 (*solid line* and *open circles*), PAO1 (*dashed line* and *solid triangles*) and 19056 mucoid (*dotted line* and *crosses*). Each *data point* represents the result from a single treatment run. *Lines* represent model-generated fits. Reproduced from (Bergen et al. 2010) with permission from the American Society for Microbiology

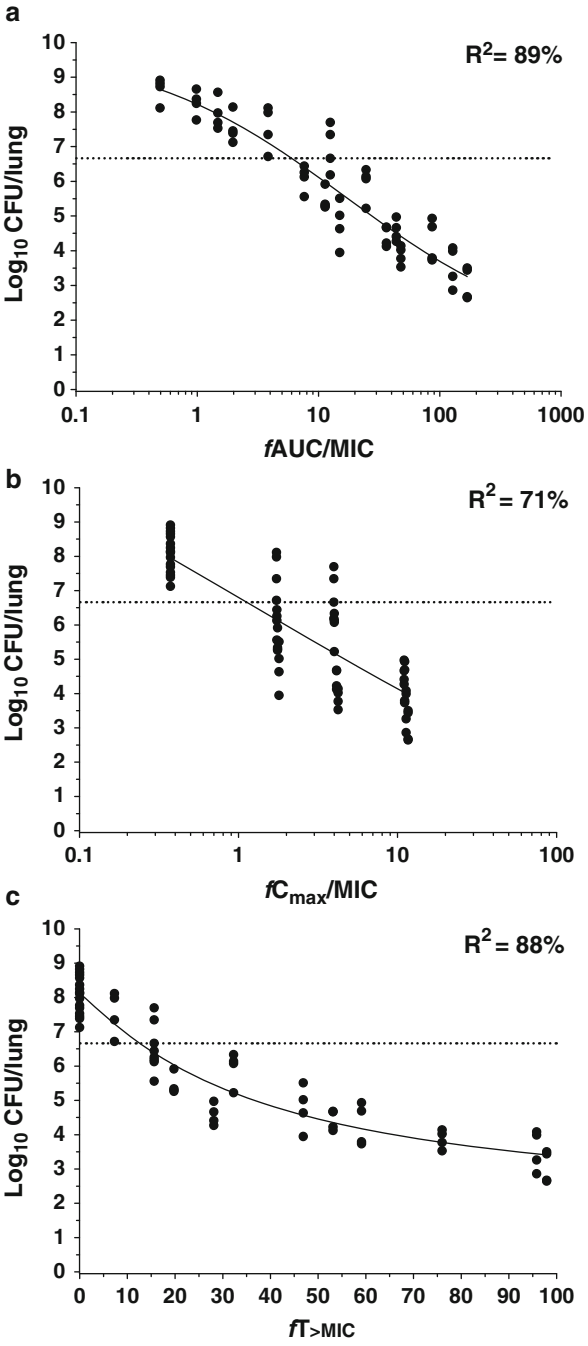

Fig. 14.4 Relationships for *P. aeruginosa* ATCC 27853 between the $\log_{10}$ CFU per lung at 24 h and the PK/PD indices (**a**) fAUC/MIC, (**b**) fC_{max}/MIC and (**c**) $fT_{>MIC}$. Each symbol represents the mean datum per mouse from two lungs. R^2 is the coefficient of determination. The *dotted line* represents the mean bacterial burden in lungs at the start of treatment. Reproduced from (Dudhani et al. 2010b, c) with permission from the American Society for Microbiology

kill were of the same order of magnitude in both the thigh and lung, although somewhat higher values were required to achieve maximal killing in the lung. Most recently, Hengzhuang et al. used a neutropenic murine lung biofilm infection model to determine the PK/PD indices for colistin most predictive of activity against planktonic and biofilm cells of a single strain of *P. aeruginosa* (Hengzhuang et al. 2012). The AUC/MIC was again the PK/PD index most closely correlated with bacterial killing of planktonic cells, whereas the AUC to minimal biofilm inhibitory concentration (MBIC) ratio (AUC/MBIC) was most predictive of killing for biofilm cells in the lung. In this study unbound colistin concentrations were not considered and AUC/MIC and AUC/MBIC values were those for total colistin. The AUC/MBIC targets identified to achieve various magnitudes of bacterial killing were substantially higher for biofilm infections than for planktonic cells. The observed differences in bacterial killing of planktonic and biofilm cells by Hengzhuang et al. (2012), as well as between thigh and lung infections by Dudhani et al. (2010b, c), suggest that dosage regimens may need to be altered depending upon the nature and/or site of infection.

Unfortunately, it is currently not possible to compare the *f*AUC/MIC targets identified from dose-fractionation studies in in vitro and animal infection models with the *f*AUC/MIC values achieved in infected patients receiving currently recommended CMS dosage regimens. Although, as discussed in the following section 'How Appropriate Are Current Dosage Regimens?', there is increasing information on the total plasma colistin concentrations occurring in CMS-treated patients, no information is currently available on unbound plasma concentrations. As such information is forthcoming it will be possible to not only assess the ability of current CMS dosage regimens to meet the above-mentioned *f*AUC/MIC targets but also to design optimised dosage regimens.

How Appropriate Are Current Dosage Regimens?

As previously discussed, colistin retains significant activity in vitro against many Gram-negative 'superbugs' and is often the only therapeutic option available to treat infections by these MDR Gram-negative pathogens (Bratu et al. 2005; Li et al. 2006b; Antoniadou et al. 2007; David and Gill 2008; Landman et al. 2008; Michalopoulos and Karatza 2010). With resistance to colistin beginning to emerge (Li et al. 2006b; Antoniadou et al. 2007; Ko et al. 2007; Johansen et al. 2008; Al-Sweih et al. 2011; Bogdanovich et al. 2011; Lee et al. 2011; Mezzatesta et al. 2011), it is imperative to administer CMS in regimens that maximise antibacterial activity and minimise resistance development, while also minimising the potential for adverse effects (e.g. nephrotoxicity). Unfortunately, a lack of information on the PK and PD of colistin and CMS has led to confusion regarding the 'optimal' dosing schedule (Nation and Li 2009). Current dosage regimens are primarily derived from manufacturers' package inserts that were developed decades ago, before an

understanding of modern PK/PD concepts. As discussed above (see section 'Inconsistent Labelling and Dose Regimens of Pharmaceutical Products'), the daily dosage recommendations for parenterally administered CMS differ substantially among products that are used in various regions of the world; this causes confusion and potentially leads to a situation of sub-optimal use.

In the product package inserts, the recommended number of CMS doses per day is 2–4 for a person with normal renal function, with 2–3 doses daily being a common regimen (Bergen et al. 2008). Once-daily regimens have also been employed despite a lack of supporting PK/PD data (Gunderson et al. 2003; Rosenvinge et al. 2005), presumably to take advantage of the concentration-dependent activity of colistin that is evident in vitro. However, in an in vitro PK/PD model that simulated human dosing regimens incorporating higher doses of CMS administered once daily, there was greater emergence of resistance in *P. aeruginosa* than occurred with a thrice-daily regimen involving essentially the same total daily dose (Bergen et al. 2008). Furthermore, a study conducted in rats involving week-long multiple-dose regimens mimicking once- and twice-daily administration in humans of the same daily dose of CMS, to achieve clinically relevant plasma colistin concentrations, resulted in a greater range and severity of renal lesions with the once-daily dosing regimen (Wallace et al. 2008a). In vitro studies have shown that the toxic effects of colistin on mammalian cells is both concentration- and time-dependent (Lewis and Lewis 2004). Finally, colistin lacks a significant postantibiotic effect (Li et al. 2001b; Owen et al. 2007; Poudyal et al. 2008; Ozbek and Senturk 2010). Thus, higher doses administered less frequently may potentially increase both nephrotoxicity and resistance development, although this remains to be confirmed in patients.

Evidence is emerging that the PK of CMS and formed colistin differs across various patient groups, and that currently used dosage regimens of CMS are likely to generate sub-optimal exposure to colistin in many patients. Li et al. reported a study in which 12 people with cystic fibrosis (age range 13–39 years, body weight range 39–68 kg, all with normal serum creatinine) were administered intravenously 1–2 million international units (IU) of colistin methanesulfonate every eight hours (equivalent to 1.83–3.50 mg of colistin base activity/kg per day) (Li et al. 2003a). The plasma CMS and colistin concentrations across a dosage interval at steady state are shown in Fig. 14.5. The peak plasma concentration of colistin was typically found in the first blood sample collected following the CMS dose and the half-life of the formed colistin was ~4 h. The range of calculated plasma colistin C_{max} at steady state was 1.2–3.1 mg/L while that for the minimum plasma concentration (C_{min}) was 0.14–1.3 mg/L, and the range of AUC over 24 h for formed colistin was 16–53 mg h/L (Li et al. 2003a). Even without consideration of protein binding, plasma colistin concentrations in many cases failed to reach the CLSI breakpoint of 2 mg/L (Clinical and Laboratory Standards Institute (CLSI) 2012) defining susceptibility to colistin for *P. aeruginosa* and *A. baumannii*, with plasma concentrations falling rapidly below this level even when achieved. It is apparent that the CMS dosage regimens employed and the resulting exposure to plasma colistin in these patients was very likely to be sub-optimal, especially with CMS monotherapy.

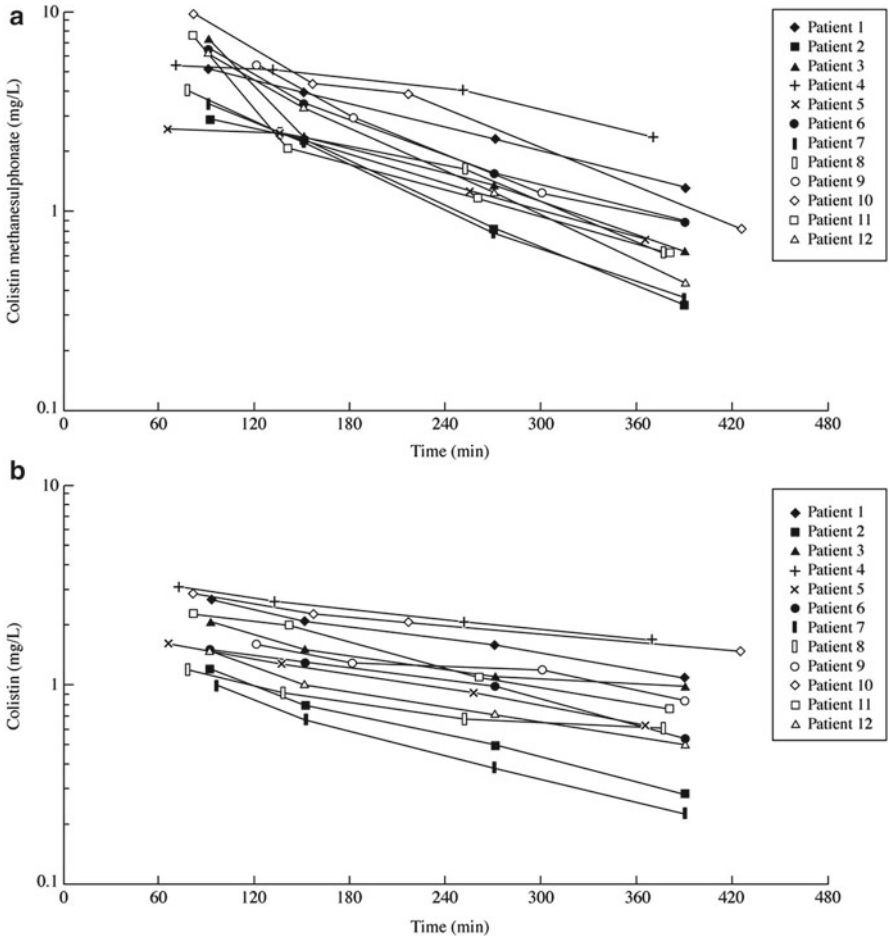

Fig. 14.5 Plasma concentrations of (**a**) colistin methanesulfonate and (**b**) colistin at steady state in 12 patients with cystic fibrosis following intravenous administration of colistin methanesulfonate 1–2 million IU every 8 h for at least 2 days. Reproduced from (Li et al. 2003a) with permission from Oxford University Press

It is increasingly obvious from studies undertaken in critically ill patients that the CMS dosage regimens used in many of these patients generate plasma colistin concentrations that are not likely to be very effective, especially when used as monotherapy (Li et al. 2005b; Markou et al. 2008; Plachouras et al. 2009; Fernandez et al. 2010; Imberti et al. 2010; Garonzik et al. 2011; Mohamed et al. 2012). Arguably, a 2005 report was the first to draw attention, based upon experimental data, to the lack of PK information for CMS and formed colistin in critically ill patients and to the lack of appropriate CMS dosage guidelines for these patients (Li et al. 2005b). Li et al. reported the disposition of CMS and formed colistin at steady state in a critically ill adult patient requiring CMS for treatment of an infection caused by MDR

P. aeruginosa. The patient had multiple organ failure requiring continuous venovenous hemodiafiltration (CVVHDF). Intravenous CMS (equivalent to 150 mg colistin base activity every 48 h) was administered as last-line therapy; the regimen was based upon the product information which suggested that in patients with renal impairment the size of the dose should be essentially maintained and the dosing interval should be increased from the normal 8–12 h. The dose actually administered to this patient was also in accord with the suggestion made, without any supporting data whatsoever, in an influential report focussing upon antibiotic dosing in critically ill patients receiving continuous renal replacement therapy (Trotman et al. 2005). Li et al. demonstrated that both CMS and colistin were cleared by CVVHDF. Importantly, total plasma concentrations of formed colistin fell below the MIC for the infecting strain ~4 h after CMS dosing. Unfortunately, 12 days after commencing CMS therapy, the patient died. Clearly, dosage adjustment for CMS in CVVHDF patients should be much more modest than that used in this patient. Subsequent studies have confirmed both CMS and colistin are efficiently cleared by intermittent hemodialysis and continuous renal replacement therapy (either CVVHDF or continuous venovenous hemofiltration) (Marchand et al. 2010a; Garonzik et al. 2011).

Makou et al. reported plasma colistin concentrations across a CMS dosage interval at least 2 days after commencing therapy (Markou et al. 2008); all patients, who were adults, had creatinine clearance >46 mL/min and 13 of the 14 patients received 2.8 million IU CMS intravenously 8 or 12 hourly (corresponding to ~270 mg colistin base activity per day). The range of C_{max} values for formed colistin was 1.15–5.14 mg/L while that for C_{min} was 0.35–1.70 mg/L; the AUC over 24 h for formed colistin ranged from 12.8 to 60.0 mg h/L. The authors expressed concern about the low plasma concentrations of colistin achieved in these patients. It was not surprising, given that all patients had moderate to good renal function, that Makou et al. were not able to discern a relationship between plasma colistin C_{max} or C_{min} and creatinine clearance. Imberti et al. reported plasma colistin concentrations across a dosage interval at least 2 days after commencing therapy in 13 adult critically ill patients with ventilator-associated pneumonia caused by Gram-negative bacteria (Imberti et al. 2010). Each patient had a creatinine clearance of >96 mL/min and received CMS two million IU intravenously 8 hourly (equivalent to ~180 mg colistin base activity per day). There was no apparent relationship between plasma colistin C_{max} (range 0.68–4.65 mg/L), C_{min} (0.23–2.43 mg/L) or AUC over 24 h (8.9–75.2 mg h/L) and creatinine clearance; as with the study of Markou et al. (2008), failure to identify a relationship between colistin PK parameters and renal function is not at all surprising given that all patients had creatinine clearance values around 100 mL/min or greater. In the study of Imberti et al. bronchoalveolar lavage (BAL) was performed 2 h after administration of a CMS dose (Imberti et al. 2010). The authors did not concentrate the BAL fluid prior to analysis to increase the sensitivity of the assay, and the colistin concentration was below the limit of detection (0.05 mg/L). It is not possible to interpret this finding because of the extensive dilution of pulmonary epithelial lining fluid (ELF) that occurs during the BAL procedure. For example, if ~100-fold dilution occurs then even if the actual concentration of colistin in BAL fluid was 0.04 mg/L this would be equivalent to 4 mg/L in ELF.

Two studies by the same research group have made a useful contribution to the understanding of important facets of the disposition of CMS and formed colistin in adult critically ill patients (Plachouras et al. 2009; Mohamed et al. 2012). In the first study, the plasma colistin concentration–time profiles observed with an intravenous CMS regimen of 3 million IU 8 hourly (equivalent to ~270 mg colistin base activity per day) in 18 critically ill patients (creatinine clearance range of 41–126 mL/min), without administration of a loading dose, revealed that total plasma colistin concentrations remained well below the MIC breakpoints for the first few doses in the regimen (Plachouras et al. 2009). Indeed, the predicted plasma colistin C_{max} from that study was 0.60 mg/L after the first dose, while plasma colistin concentrations ≥2 mg/L were not achieved until approximately 44 h after commencing therapy; the typical plasma colistin C_{max} at steady state was estimated to be 2.3 mg/L. Even at steady state, the plasma concentrations of formed colistin were below the MIC breakpoint in many patients, without consideration of plasma protein binding. In a follow-up study, the same group reported clinical PK data on a further ten critically ill patients (creatinine clearance range of 24.9–214.3 mL/min; intravenous CMS maintenance doses of 1–3 million IU 8 hourly) (Mohamed et al. 2012). The PK data were analysed simultaneously with the data from the original study (Plachouras et al. 2009). Once again, steady-state plasma colistin concentrations were not achieved for 2–3 days, were low (the average colistin C_{max} at steady-state was 2.3 mg/L) and a large fraction of the patients had plasma colistin concentrations below the MIC breakpoint of 2 mg/L (Mohamed et al. 2012). Importantly, delayed initiation of appropriate antimicrobial therapy is associated with increased mortality in critically ill patients (Kumar et al. 2006; Luna et al. 2006), and low colistin concentrations have been associated with the amplification of colistin-resistant subpopulations (Tan et al. 2007; Bergen et al. 2008, 2011a, b; Poudyal et al. 2008; Bulitta et al. 2010; Dudhani et al. 2010b). Mathematical modelling by Bulitta et al. predicted colistin regimens with a large colistin exposure during the first ~12 h may be beneficial, providing enough net killing such that the immune system may be able to eradicate any remaining colistin-resistant cells (Bulitta et al. 2010). Thus, it is evident from the data presented by Plachouras et al. and Mohamed et al. that therapy with CMS should commence with a loading dose, which was suggested by the authors (Plachouras et al. 2009; Mohamed et al. 2012). Because of the potential for nephrotoxicity, it is suggested that the loading dose for an adult should not exceed 300 mg colistin base activity, with the first maintenance dose administered 24 h later (Garonzik et al. 2011). Because there were only 28 patients in total in the two studies reported by Plachouras et al. and Mohamed et al., with all but one patient having a creatinine clearance of >41 mL/min, it was not possible for these investigators to identify any relationships between the CMS or colistin kinetics and renal function (Plachouras et al. 2009; Mohamed et al. 2012).

The impact of renal function in critically ill patients on the disposition of CMS and formed colistin is evident from the largest pharmacokinetic study to date involving 105 patients, 89 of whom had very diverse renal function (creatinine clearance 3–169 mL/min/1.73 m^2) but were not receiving renal support, 12 who were receiving intermittent hemodialysis and 4 who were recipients of continuous renal

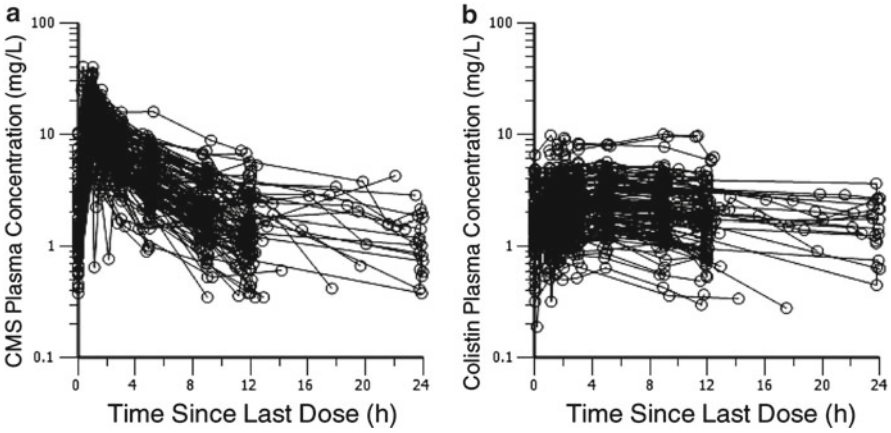

Fig. 14.6 Steady-state plasma concentration-time profiles of (**a**) colistin methanesulfonate and (**b**) formed colistin in 105 critically ill patients (89 not on renal replacement, 12 on intermittent hemodialysis and 4 on continuous renal replacement therapy). The physician-selected daily dose ranged from 75 to 410 mg colistin base activity; the dosage intervals ranged from 8 to 24 h and hence the inter-dosing blood sampling interval spanned the same range. Reproduced from (Garonzik et al. 2011) with permission from the American Society for Microbiology

replacement therapy (Garonzik et al. 2011). The plasma concentration-time profiles of CMS and formed colistin across a dosage interval at steady state in the 105 patients who were not receiving renal support are presented in Fig. 14.6. It is evident that within each patient there was generally little fluctuation in the plasma colistin concentrations across a dosage interval, consistent with a protracted half-life for formed colistin in these very sick patients. The CMS dosage regimens administered to these patients (median daily dose across the 105 patients was 200 mg colistin base activity; range 75–410 mg colistin base activity per day) achieved average steady-state plasma colistin concentrations of 0.48–9.38 mg/L (median, 2.36 mg/L; Fig. 14.6), corresponding to a range of AUC over 24 h of 11.5–225 mg h/L. That is, the ~5.5-fold range of CMS daily doses generated a ~20-fold range of exposure to colistin in plasma. The importance of renal function as a determinant of the plasma colistin concentrations achieved from a given daily dose of CMS can begin to be appreciated from the data presented in Fig. 14.7. Indeed, population PK modelling revealed that creatinine clearance was an important covariate for the clearance of CMS and the apparent clearance of colistin. That the clearance of CMS correlated with renal function is not surprising given that CMS is predominantly cleared by renal excretion. However, the fact that creatinine clearance was a covariate for the apparent clearance of formed colistin may, at first thought, seem rather surprising because colistin is mainly excreted by non-renal mechanisms (see section 'Pharmacokinetics, Pharmacodynamics and Pharmacokinetic/Pharmacodynamic Relationships'). The explanation lies in the schema shown in Fig. 14.2. In patients with relatively normal renal function, only a very small fraction of an administered dose of CMS is converted to colistin because the renal clearance of CMS is

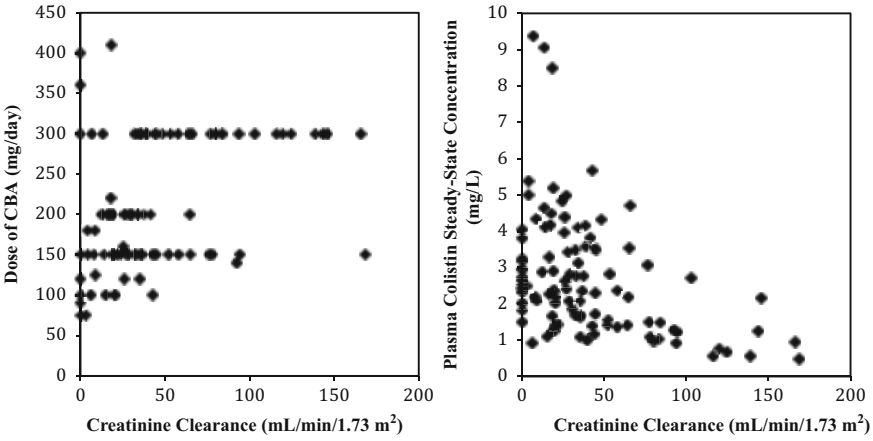

Fig. 14.7 Relationship of (**a**) physician-selected daily dose of colistin base activity (CBA) and (**b**) the resultant average steady-state plasma colistin concentration with creatinine clearance in 105 critically ill patients. Reproduced from (Garonzik et al. 2011) with permission from the American Society for Microbiology

substantially greater than the clearance for the formation of colistin from CMS. However, in patients with substantial reductions in kidney function, the renal clearance of CMS declines and consequently a greater fraction of the administered dose of CMS is converted to colistin. Thus, the apparent clearance of colistin correlates with creatinine clearance, leading to at least two important practical consequences. First, it is evident that in patients with moderate to good renal function, administration of a daily dose of colistin base activity at the upper limit of the current product-recommended dose range (300 mg colistin base activity per day) (Coly-Mycin 2005) was not able to generate plasma colistin concentrations that would be expected to be reliably efficacious (Fig. 14.6). The second practical consequence is that reduction of the daily dose of CMS is required as renal function declines, in patients who are not receiving renal support with either intermittent hemodialysis or continuous renal replacement therapy. In agreement with previous case reports concerning critically ill patients on intermittent hemodialysis Marchand et al. (2010a) or continuous renal replacement therapy (Li et al. 2005b), both CMS and colistin were shown to have relatively efficient extracorporeal clearance in the 12 and 4 patients, respectively, who were receiving these forms of renal support (Garonzik et al. 2011). A very important practical outcome of this study was the generation of the first scientifically based CMS-dosing algorithms for patients with a large range of renal function, including patients on intermittent hemodialysis or continuous renal replacement therapy; the algorithms allowed calculation of the CMS loading and maintenance doses required to achieve a desired target average steady-state plasma concentration for colistin. Overall, the observations from this study (Fig. 14.7) are a cause for significant concern, suggesting suboptimal exposure to formed colistin with current CMS dosage regimens, particularly when one or more

of the following applies: (1) the patient has moderate to good renal function, in which case it is unlikely that even a CMS daily dose at the upper limit of the product-recommended dose range will generate plasma colistin exposure likely to be reliably efficacious; (2) MIC of the infecting strain is in the upper range (i.e. 2 mg/L) of the susceptibility region for colistin; and (3) the infection is associated with high bacterial numbers. Under these circumstances, the most appropriate approach is likely to be therapy with a rationally selected colistin combination regimen.

Conclusions

The last several years have seen significant advances in unravelling of key aspects of the pharmacokinetics and pharmacodynamics of colistin and the relationship between the pharmacokinetics and pharmacodynamics, resulting for the first time in the generation of scientifically based dosing algorithms for CMS. As both preclinical and clinical investigations continue there will be further steps towards understanding how to optimise the administration of colistin methanesulfonate. The future incorporation of human PK/PD data into dosing algorithms, including information on colistin plasma protein binding and endpoints such as clinical cure, bacteriological eradication and the development of resistance will be very important. In addition, studies examining colistin monotherapy versus combination therapy will facilitate further optimization of colistin therapy in the various categories of patients who now require this important last-line antibiotic against Gram-negative pathogens.

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Chapter 15
Daptomycin: Pharmacokinetic, Pharmacodynamic, and Dose Optimization

Céline Vidaillac and Michael J. Rybak

Abstract Daptomycin is a parenteral cyclic lipopeptide antibiotic approved for the treatment of infections caused by Gram-positive pathogens including multidrug resistant *Staphylococci* and *Enterococci* sp. In addition to its unique mechanism of action daptomycin also displays unique pharmacological properties, with a linear pharmacokinetic profile, a good tolerability, a rapid and concentration-dependent bactericidal effect, and a low clinical resistance rate. Daptomycin dosages up to 12 mg/kg/day and antimicrobial combinations have been recently suggested to increase and preserve the effectiveness of daptomycin from the development of resistance. This chapter aims to provide medical and scientific readers with main pharmacokinetic and pharmacodynamic properties of daptomycin and discuss available and potential strategies developed to optimize its clinical use.

Keywords Daptomycin • Pharmacokinetic properties • Pharmacodynamic properties • Dose optimization

Introduction

Daptomycin (Cubicin®, Cubist Pharmaceuticals, Lexington, MA) is a cyclic lipopeptide antibiotic that has been developed in the late 1970s as a potentially more active and safer option to vancomycin. Although considered as one of the most potent antibiotics available to treat Gram-positive infections, the early development of daptomycin has been challenged by dose optimization issues. Unexpected

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treatment failures and increased skeletal muscle toxicity resulted in the rapid discontinuation of preclinical studies (Hawkey 2008). It is only several years later that the use of higher doses (equal or greater than 4 mg/kg/day) and a once-daily administration contributed to restore daptomycin to its former glory, improving its antimicrobial activity and minimizing induced myopathy (Eliopoulos et al. 1986; Van der Auwera 1989; Oleson et al. 2000).

Based on its favorable pharmacokinetic/pharmacodynamic (PK/PD) properties, daptomycin is often described as the ideal antibiotic. In addition to a rapid concentration-dependent bactericidal effect, daptomycin is also well tolerated, demonstrates no significant drug interactions, has a linear PK profile and a long postantibiotic effect (PAE) (Tedesco and Rybak 2004). Daptomycin has been approved by the Food and Drug Administration (FDA) in 2003 for the treatment of *Staphylococcus aureus* bloodstream infections including those with right-sided infective endocarditis (IE), as well as complicated skin and skin structure infections (cSSSI) caused by susceptible and resistant *S. aureus* (MSSA and MRSA), *Streptococcus* spp. and vancomycin-susceptible *Enterococcus faecalis*. Four years later, daptomycin was approved in Europe for the same indications. Recommended regimens are 4 and 6 mg/kg/day over a 30-min intravenous infusion (IV) for respectively complicated skin and skin structure infections (cSSSI) and bloodstream infections caused by susceptible and resistant *S. aureus* (MSSA and MRSA), *Streptococcus* spp., and vancomycin-susceptible *Enterococcus faecalis*. Daptomycin clinical failures have been rarely reported, and mainly observed during the treatment of complicated infections with vancomycin-resistant *Enterococci* (VRE) or secondary to vancomycin therapy in deep-seated infections, such as osteomyelitis requiring surgical debridement, drainage, or infected hardware involving *S. aureus* organisms (Lee et al. 1991; Marty et al. 2006; Segreti et al. 2006; Lesho et al. 2006; Long et al. 2005; Skiest 2006; Hirschwerk et al. 2006).

As a value to practicing physicians and infectious diseases pharmacists, we offer a large review of in vitro and in vivo pharmacokinetic (PK) and pharmacodynamic (PD) data on daptomycin and discuss available and potential strategies developed to optimize its clinical use. Since PK/PD relationships represent a key link between optimal drug dosing and clinical outcomes, a better understanding of how the body interacts with daptomycin and how the drug exposure reflects daptomycin activity is crucial to optimize patient outcomes and reduce the risk of resistance.

Pharmacokinetics

General Pharmacokinetic Properties

Daptomycin is administered intravenously with a standard infusion time of 30 min to minimize infusion-related adverse effects. Despite efforts made to develop an oral formulation, the option was rapidly abandoned due to the poor oral absorption of daptomycin. Other routes of administration (intramuscular, intraventricular,

intraperitoneal, and intrathecal infusions) were also investigated, but limited data are available (Huen et al. 2009; Elvy et al. 2008; Albin 2009).

Pharmacokinetic properties of daptomycin can be characterized using a two-compartment pharmacokinetic profile (Dvorchik et al. 2004). Table 15.1 summarizes major PK parameters observed following administration of single and multiple doses up to 12 mg/kg/day. In patients with normal creatinine clearance, daptomycin displays an α-distribution phase of approximatively 7 min and a β-elimination half-life of 8–9 h, allowing for a once-daily administration (Dvorchik et al. 2003). Daptomycin primary route of excretion are the kidneys with 78 % urinary recovery (including 50 % of unchanged and active compound), whereas the fecal excretion is only minimal (approximately 6 %). Renal function, sex, and body temperature influence the clearance of daptomycin with renal function being the most significant inter-individual variable (Dvorchik et al. 2004). Other variables including associated comorbidities (diabetes, hypertension, congestive heart failure, and edema) and coadministration of medications do not correlate with daptomycin clearance. Cautions are, however, warranted with tobramycin, warfarin, and simvastatin since interactions of daptomycin with these drugs remain unknown. Daptomycin binds reversibly to plasma proteins and irreversibly to bacterial membranes at approximately 90–94 % in a concentration-independent manner. Of interest, since protein-bound daptomycin remains bioavailable (constant of dissociation of albumin of 90.3 μM), the percentage of free drug is not considered as a good predictor for daptomycin efficacy (Eisenstein 2004). The volume of distribution of daptomycin is linearly correlated to the body weight and is usually low (approximately 0.1 L/kg), which implies a poor tissue penetration and a substantial retention in the blood compartment due to its high protein binding (Dvorchik et al. 2004) (Table 15.2). Concentrations of daptomycin vary between 2 and 75 % relative to serum, the higher penetration being observed in kidneys and the lower penetration in bones, CSF, and lung (Steenbergen et al. 2005; Kullar et al. 2011). Of note, daptomycin seems to moderately cross the blood–brain and placental barriers (Cottagnoud et al. 2004; Riser et al. 2010; Mader and Adams 1989; Alder et al. 2003). In contrast and of interest, daptomycin penetrates into blood-clot tissues fairly well (up to 75 %) following a homogeneous distribution pattern (Michiels and Bergeron 1996; Allen et al. 2003; Caron et al. 1992).

Special Populations

Obesity

Since obesity may affect drug distribution and elimination, daptomycin PK has been investigated in overweight subjects (Dvorchik et al. 2004; Pai et al. 2007). Dvorchik and colleagues studied two groups of six moderately (BMI = 25–39.9 kg/cm^2) and six morbidly (BMI ≥ 40 kg/cm^2) obese patients matched with 12 nonobese healthy patients, using sex, age, ethnic origins, and renal function (estimated by the clearance) as criteria. All patients received 4 mg/kg total body weight by intravenous infusion over 30 min. Daptomycin C_{max} and $AUC_{0–24\,h}$ values were found to be

Table 15.1 Daptomycin PK parameters related to the dose on day 1 and at steady state: comparison of a standard 30 min infusion (Dvorchik et al. 2003, 2004; Woodworth et al. 1992)

		Dose (mg/kg/day)									
	PK parameters	0.5	1	1.5	2	3	4	6	8	10	12
Day1	C_{max} (mg/L)	6.37 ± 1.31	13.02 ± 1.69	17.43 ± 2.81	23.66 ± 6.1	41.55 ± 7.48	53.43 ± 13.6	88.05 ± 34.1	111.26 ± 20	129.7	164.8
	AUC (mg h/L)	41.8 ± 3.83	93.4 ± 16.8	139 ± 20	213 ± 169.2	329 ± 46.9	403.5 ± 173.7	677.33 ± 241.8	950 ± 514	1,013.5	1,269.2
	$T_{1/2}$ (h)	5.78	6.42	9.37	9.72	8.35	7.98	7.79	8.45	8.4	7.8
	Clwp (mL h/kg)	5.46 ± 0.42	5.94 ± 0.78	4.68 ± 1.26	7.47 ± 4.5	3.1 ± 1.26	6.78 ± 5.56	7.31 ± 6.49	8.65 ± 4.0	9.9	10.0
	V (mL/kg)	101 ± 6	103 ± 18	150 ± 16	140 ± 66	113 ± 17	116.5 ± 9	107.3 ± 53	101 ± 17.9	117.2	111.1
Steady state	C_{max} (mg/L)	ND	ND	ND	ND	ND	57.8 ± 3	96.25 ± 5.15	128.15 ± 18.1	141.1	183.7
	AUC_{0-inf} (mg h/L)	ND	ND	ND	ND	ND	494 ± 75	689.4 ± 105.2	994 ± 131.9	1,038.8	1,277.4
	$T_{1/2}$ (h)	ND	ND	ND	ND	ND	8.1 ± 1	8.4 ± 1.6	8.65 ± 2	7.9	7.7
	Clwp (mL h/kg)	ND	ND	ND	ND	ND	8.3 ± 1.3	8.6 ± 0.77	8.1 ± 0.94	8.8	9.0
	V (mL/kg)	ND	ND	ND	ND	ND	96 ± 9	102.7 ± 11.4	96.65 ± 7.83	98.3	97.6

ND not determined

Table 15.2 Daptomycin pharmacokinetics and tissue penetration

Tissue	Species	Dose	Concentration	% Relative to serum	References
		mg/kg/day	C_{max}		
Soft tissue	Healthy volunteers	4	3.8 ± 1.4 mg/L	6.1	Chung et al. (2008)
	Diabetic	4	4.3 ± 3.3 mg/L	6.3	
Inflammatory fluid	Human	4	27.6 ± 9.5 mg/L	68.4	Wise et al. (2002)
CSF	Rabbit	15 (similar to 6)	5.2 mg/L	6.011	Cottagnoud et al. (2004)
Blood clot tissue	Rabbit	10 bid	11.6 ± 10.8 mg/kg	17.5	Caron et al. (1992)
		40 bid	22.5 ± 1.4 mg/kg	24.2	
	In vitro SEV model	6	76.5 ± 10.8 mg/kg	74.7	Allen et al. (2003)
Bones	Rabbit	4 mg/kg	~0.5 mg/L	1.3	Mader and Adams (1989)
Lung	Mouse, rat	–	5 mg/L	9.3	Alder et al. (2003)
BAL-ELF	Mouse, rat, sheep	–	1 mg/L	2	Alder et al. (2003)
Peritoneal tissue chamber	Rat	30	11.8 mg/L	35.1	Vaudaux et al. (2003)

ND not determined

≈30 % higher in the obese groups compared to the nonobese group, and values proportionally increased with the volume of distribution and the clearance (Dvorchik et al. 2004; Dvorchik and Damphousse 2005). These results are however in contradiction with a more recent study from Pai and colleagues evaluating daptomycin PK from two groups of seven morbidly obese and seven normal-weight healthy patients, using the same matching criteria (Pai et al. 2007). In an attempt to improve the accuracy of the renal function estimates, the authors followed the recommendations of the National Kidney Foundation. They used a measurement and an estimation of the glomerular function rate as well as an estimation of the creatinine clearance, using multiple body size descriptors in the Cockcroft–Gault equation. Although they also found an increase in the C_{max} and $AUC_{0–24\,h}$ values (≈60 %) for the obese group, they did not observe any statistical differences between the two groups regarding the volume of distribution and the clearance. They concluded that the difference observed in the overweighted group was only a function of the total drug administrated. No adjustment in daptomycin dose or dose regimen is therefore recommended based on the obesity pattern at this moment.

Patients with Renal Dysfunction

Daptomycin is recommended for patients with documented Gram-positive infections (except VRE-related and pulmonary infections) and receiving high-flux hemodialysis (HD) (Pai and Pai 2006). However, since its elimination half-life is dramatically increased in patients with a $Cl_{cr} \leq 40$ mL/min (reported up to 30 h in patients under HD), dose adjustment (30 min infusion every 48 h) is required for patients with renal impairment or on HD (Dvorchik et al. 2004). In addition, in case of low- and high-permeability dialyzers, adjustment of intradialytic dosages at 7 or 9 mg/kg (for 6 mg/kg/day) should be considered (Salama et al. 2009).

Young and Geriatric Populations

Although daptomycin is not FDA approved for neonates, children, and young adolescents populations (<18 years old), some preliminary PK data and observations have been reported (Abdel-Rahman et al. 2008; Ardura et al. 2007). An inverse linear correlation between clearance and age has been observed, suggesting that higher dosages than those administered to adults may be required in the pediatric population. Indeed, in a study involving two groups of 12 younger (18–30 years old) and older (≥75 years old) patients treated with daptomycin, drug exposure was reported to be 58 % higher in the older population as a result of a decrease clearance in this group (Dvorchik et al. 2004). In contrast, C_{max} and volume of distribution were found unchanged and differences observed with elderly were ascribed to changes in renal function as a function of age. Since investigations are still warranted for the use of daptomycin in these populations, drug and creatinine phosphokinase concentrations need to be monitored to ensure appropriate drug exposure while avoiding toxicity.

Other Patient Populations

Patients with thermal burn injuries usually present physiological changes affecting drug pharmacokinetics. In an open-label study enrolling nine patients with burn injuries affecting more than 18 % of body surface, daptomycin PK parameters were significantly different from those reported in nonburn patients (Mohr et al. 2008). The peak concentration and the $AUC_{0-\infty}$ in burn patients were substantially reduced (44 and 47 %, respectively), whereas the volume of distribution and the clearance were highly increased (64 and 77 %, respectively). Data reported in this study also supported a clearance of daptomycin through the burn wounds. Since the AUC is decreased by about 47 % with a standard dose of 6 mg/kg/day, dose adjustments are recommended in these patients. In order to achieve drug exposures similar to those observed in healthy volunteers an increase up to 12 mg/kg/day is recommended (Dvorchik et al. 2003; Mohr et al. 2008).

No significant difference in daptomycin PK was observed in healthy patients, HIV positive patients, and patients with liver function abnormalities (Dvorchik 2004; Pryka et al. 1990). No dose adjustments are recommended at this time in patients with hepatic impairment or positive for human immunodeficiency virus; however, further investigations with larger sample sizes are warranted.

Pharmacodynamics

In Vitro and In Vivo Assessment of Daptomycin PD Parameters

Daptomycin exhibits the most rapid and potent bactericidal activity amongst all antimicrobial used as monotherapy against MDR Gram-positive pathogens (Allen et al. 2003; Fuchs et al. 2002; Aeschlimann et al. 2000) Of interest, daptomycin has been shown to be minimally affected by inoculum size (LaPlante and Rybak 2004a) and metabolic stage (Mascio et al. 2007) and to rapidly penetrate the complex matrix of staphylococcal biofilm (Stewart et al. 2009; Kim et al. 2009). Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and postantibiotic effects (PAEs) are quantifiable parameters that provide information on the susceptibility and potency of antibiotics. Daptomycin MIC_{90} values are typically 0.25–0.5, 1, and 0.25 mg/L for *Staphylococci* spp., vancomycin-susceptible *Enterococci* spp., and group B *Streptococci* spp., respectively (Sader et al. 2005). Daptomycin also exhibits a prolonged and dose-dependent PAEs (from 1.1 to 6.2 h against *S. aureus* and in the 1 to 2.5 h range against *S. pneumoniae*) (Hanberger et al. 1991; Safdar et al. 2004; Pankuch et al. 2003). Unfortunately, the ability of these PD parameters to predict clinical efficacy is limited since they do not take into account any interactions of the drug or its penetration at the site of infection. Pharmacodynamic/pharmacokinetic (PK/PD) index parameters to better capture this include the duration of time the unbound antibiotic concentration exceeds the minimum inhibitory concentration ($T>$MIC), the ratio of maximum serum antibiotic concentration (C_{max}) to the MIC (C_{max}/MIC), and the ratio of the area under the concentration–time curve during a 24-h dosing interval ($AUC_{0–24}$) to the MIC ($AUC_{0–24}$/MIC). These ratios have the advantage of associating antibiotic concentration with bacterial killing or growth inhibition properties. The $AUC_{0–24\ h}$/MIC and C_{max}/MIC ratios were identified as the best PD indices to predict daptomycin antimicrobial efficacy against *S. aureus*, *S. pneumoniae*, and *E. faecium* isolates (Table 15.3) (Safdar et al. 2004; Louie et al. 2001). Using an immunocompromised thigh infection model and a bacterial inoculum of 10^7 CFU/mL, Dandekar et al. reported that a *f*AUC/MIC ratio of 171–442 and 38–157 were required to achieve bactericidal activity against MRSA and *Enterococcus* spp., respectively (Dandekar et al. 2003). Similar values were recently reported by Torrico et al. with slight variations attributed to changes in the vancomycin-susceptibility profile (Torrico et al. 2011).

Table 15.3 AUC/MIC and C_{max}/MIC ratios required to produce bacteriostatic and killing effects over 24 h (Safdar et al. 2004)

Organism	MIC (mg/L)	Static dose (mg/kg/24 h)	$AUC_{0-24\,h}$/MIC ratio			C_{max}/MIC ratio		
			Static dose	1 log kill	2 log kill	Static dose	1 log kill	2 log kill
S. aureus ($n=4$)	0.5	23.45 ± 3.03	438 ± 67	666 ± 87	1.061 ± 296	70.6 ± 15.6	129 ± 24.1	255 ± 104
S. pneumoniae ($n=8$)	0.125–0.25	2.99 ± 1.44	160 ± 51	290 ± 121	498 ± 131	24 ± 7.6	42.1 ± 17.2	73.9 ± 34.2
E. faecium ($n=2$)	2	0.28 ± 0.08	1.31 ± 0.36	18.97 ± 14.8	ND	0.19 ± 0.06	2.83 ± 2.22	ND

ND not determinated

Combinations Therapy

Because of potential for increased toxicity and higher cost, combination therapy remains a controversial therapeutic strategy employed by clinicians as an alternative to treat life-threatening infections caused by multidrug resistant pathogens in seriously ill patients. In terms of PK/PD benefits, it also appears more difficult to predict the in vivo efficacy of antimicrobial agents administered as combination therapy compared with monotherapy for which PK/PD indices may be used. In 1999, Mouton et al. investigated the hypothesis that the PD index used to predict in vivo efficacy of a single antibiotic would be similar to that used when administrated in combination (Mouton et al. 1999). Using multiple regression analysis, they demonstrated that the two PD indices that explain the activity of ticarcillin and tobramycin taken separately were also able to describe most of the effects observed with the combination. In contrast, using ciprofloxacin and netilmicin for which in vivo efficacy is predicted by the same parameter (AUC/MIC), the best index to explain the effect of the combination was the sum of the AUC/MIC values. Of interest, they reported that for daptomycin the use of two agents given as a once-daily dose resulted in variable results and postulated that this was related to the low concentrations at the end of the dosing regimen. They concluded that both total dose (i.e., AUC) and time above MIC should be considered for daptomycin combinations (Mouton et al. 1999).

Experimental In Vitro and In Vivo PK/PD Models

Several studies using in vitro PK/PD models have investigated the potential for synergy of daptomycin in combination with rifampin, gentamicin, cefepime, tigecycline, arbekacin, linezolid, or trimethoprim/sulfamethoxazole against *S. aureus* and *Enterococci* clinical isolates (Table 15.4) (Steenbergen et al. 2005). Some combinations including daptomycin plus gentamicin (5 mg/kg/day) or rifampicin (300 mg q8h) were beneficial by reducing the time to achieve bactericidal effect against *S. aureus* and *Enterococci* isolates (LaPlante and Rybak 2004b; Chan et al. 2008; Miro et al. 2009; Steed et al. 2010; Sakoulas et al. 2003). Other combinations were beneficial by resulting in a synergistic effect under specific conditions such as high inoculum (daptomycin plus arbekacin and more recently trimethoprim/sulfomethoxazole) (Steed and Rybak 2010) or presence of biofilm and combination daptomycin and clarithromycin or rifampin (Parra-Ruiz et al. 2010).

Clinical Case Reports

Only two papers available online reported on clinical cases of VRE endocarditis treated with daptomycin plus tigecycline or gentamicin and ampicillin (Arias et al.

Table 15.4 Daptomycin activity and potential for resistance alone or in combination against *S. aureus* and *Enterococcal* isolates

Species	DAP MIC (mg/L)	DAP regimen	Combined drug (dose)	LOT	Starting Inoculum	Effects of the combination vs. DAP alone			Emergence of non susceptible isolates		References
						Faster bactericidal activity	Increase activity	Synergy	Combination	DAP	
MSSA	0.25	6 mg/kg/day	GEN 5 mg/kg q24h GEN 1 mg/kg q8h	4 days	≈9 $\log_{10}$ CFU/g of SEV	Yes	No	No	No	No	Tsuji and Rybak (2005)
		8 mg/kg/day	GEN 5 mg/kg q24h GEN 1 mg/kg q8h								
	0.25	6 mg/kg/day	GEN 5 mg/kg q24h	3 days	≈9 $\log_{10}$ CFU/g of SEV	Yes	No	No	No	No	LaPlante and Rybak (2004b)
MRSA	1	40 mg/kg/day s.c.	RIF 25 mg/kg/day s.c.	5 days max	≈6 $\log_{10}$ CFU/g	Not mentioned	Yes	No	No	No	Sakoulas et al. (2003)
	0.25	25 mg/kg/b.i.d s.c.	RIF 6 mg/kg i.m.	5 days max		Not mentioned	No	No	No	No	Voorn et al. (1994)
	0.125–0.5	3 mg/kg/day 4 mg/kg/day 6 mg/kg/day	ARB 100 mg q12h	2 days	≈6 $\log_{10}$ CFU/mL	No	Yes	Yes	No	No	Akins and Rybak (2000)

	0.06–0.125	6 mg/kg/day	CEF 2 g q8h CEF 2 g q12h CEF CI (2 g + 4 g q24h)	2 days	≈6 $\log_{10}$ CFU/mL	Not mentioned	No	No	No	No	Huang and Rybak (2006)
	0.25	6 mg/kg/day	GEN 5 mg/kg q24h GEN 1 mg/kg q8h	4 days	≈9 $\log_{10}$ CFU/g of SEV	Yes	No	No	No	No	Tsuji and Rybak (2005)
		8 mg/kg/day	GEN 5 mg/kg q24h GEN 1 mg/kg q8h								
	0.5	6 mg/kg/day	RIF 300 mg q8h i.v.	2 days	≈6 $\log_{10}$ CFU/g of SEV	Not mentioned	No	No	No	Yes	Miro et al. (2009)
			GEN 1 mg/kg q8h i.v.							No	
	0.25	6 mg/kg/day	GEN 5 mg/kg q24h	3 days	≈9 $\log_{10}$ CFU/g of SEV	Yes	No	No	No	No	LaPlante and Rybak (2004b)
	4	6 mg/kg/day	LIN (600 mg q12h) TMP/SXT (160/800 mg q12h) CEF (2 g q12h)	3 days	≈9 $\log_{10}$ CFU/g of SEV	Yes	No	No	No	No	Steed and Rybak (2010)
E. faecalis	1	10 mg/kg/day	GEN 0.6 mg q12 h i.m.	6 weeks	≈6 $\log_{10}$ CFU/g of kidney tissues	No	Yes	No	Not mentioned	Not mentioned	Sapico et al. (1988)
	4	20 mg/kg/b.i.d s.c.	FOS 500 mg/kg/day	5 days	≈7 $\log_{10}$ CFU/g of SEV	Not mentioned	Yes	No	Not mentioned	Not mentioned	Rice et al. (1992)
E. faecium	2	10 mg/kg q12h 12 mg/kg q12h	GEN 6 mg/kg q12h	5 days	≈8 $\log_{10}$ CFU/g of SEV	Yes	No Yes	No Yes	Not mentioned	Not mentioned	Caron et al. (1992)

MSSA methicillin-susceptible *S. aureus*, *MRSA* methicillin-resistant *S. aureus*, *SEV* simulated endocardial vegetations, *DAP* daptomycin, *GEN* gentamicin, *RIF* rifampicin, *LIN* linezolid, *TMP/SFX* trimethoprim/sulfamethoxazole, *CEF* cefepime, *FOS* fosfomycin, *CI* continuous infusion

2007; Jenkins 2007). In the first case report, a 62-year-old patient presenting with signs and symptoms of IE, received vancomycin (1 g IV q12h) followed by linezolid (600 mg orally q12h) after which clinicians switched to daptomycin (6 mg/kg q48h) plus tigecycline (100 mg IV followed by 50 mg q12h). Blood cultures became negative after 10 days of daptomycin plus tigecycline combination therapy (Jenkins 2007). In the second case report, a 60-year-old diabetic man was treated with daptomycin 6 mg/kg/day for VRE IE (Arias et al. 2007). After 3 days of negative blood cultures, the patient was discharged to complete a 6-week course of therapy. During week 5, the patient examination revealed persistence of vegetation and blood cultures yielded α-*Streptococcus* and vancomycin-susceptible *Enterococci* (daptomycin and vancomycin MIC of 2 mg/L). Although blood cultures became negative after 5 days of vancomycin (15 mg/kg q12h) plus gentamicin (1 mg/kg q12h), VRE isolates were recovered from the blood 2 weeks after initiation of vancomycin therapy. The infection was finally resolved by a combination of daptomycin (8 mg/kg/day) plus gentamicin (1 mg/kg/day) and ampicillin (16 g/day). Analysis of the different isolates by pulse field gel electrophoresis indicated that the *Enterococci* isolates were the same as cultured before, demonstrating the loss of the *vanA* gene cluster under daptomycin therapy or the selection of subpopulations that did not carry the *vanA* genotype (Arias et al. 2007).

Combination or No Combination Therapy?

Combination therapy is a controversial therapeutic strategy employed to boost the bactericidal effect of the therapeutic intervention, extend the spectrum of activity, and reduce the likelihood of development of resistance. It is difficult to predict the PD benefit of this strategy applied to daptomycin, since the complete mechanism is complex and not fully understood (Steenbergen et al. 2005). A shortened time to achieve cidal activity and a protective effect against nephrotoxicity of aminoglycoside (due to lower dose) may be however of benefit, especially in patients presenting serious signs and symptoms of infections (Beauchamp et al. 1990, 1994; Thibault et al. 1995). The recent MRSA guidelines published by the Infectious Diseases Society of America recommend a high-dose daptomycin regimen of 10 mg/kg/day in combination with either rifampin, gentamicin, or trimethoprim/sulfamethoxazole for patients with persistent MRSA bacteremia failing vancomycin therapy (Liu et al. 2011). Judicious selection of antimicrobials used in combination with daptomycin should also be performed. For example, rifampin has been demonstrated to be an inducer of the P-glycoprotein (or P-gp), a multidrug transporter involved in the efflux of xenobiotics and metabolites from cells into the intestinal lumen, bile, and urine. Daptomycin has been reported to be a substrate for this efflux pump that is present at the surface of eukaryotic cells and may play a role in daptomycin intracellular activity or in daptomycin elimination (Lemaire et al. 2007; Baietto et al. 2010).

Resistance

PK and PD parameters play a key role in the low level of resistance mediated by either alteration of the cell wall (β-lactams, vancomycin), activation of efflux pumps, or emergence of point mutations in the target (fluoroquinolones, β-lactams). The microbiological definition of reduced susceptibility to daptomycin has not been established yet and the complete mechanism remains unclear. Currently, *S. aureus* isolates with an MIC value > 1 mg/L are considered nonsusceptible. The first nonsusceptible strain was isolated in the late 1990s during a clinical trial involving intravenous drug users (Eli Lilly 1990) and low doses of daptomycin. In vitro investigations performed on the parent nonsusceptible organisms (SA-675 and SA-684) revealed that subinhibitory concentrations of daptomycin resulted in the emergence of populations with heterogeneous susceptibility and alteration of cell wall thickness that could be prevented by using higher dosages (6 vs. 10 mg/kg/day) (Rose et al. 2008; Kaatz et al. 1990, 2006). An extensive review of the literature by Falagas and colleagues in 2007 reported seven daptomycin nonsusceptible isolates over 60 clinical cases of endocarditis and bacteremia (19 patients were treated for endocarditis and 41 for bacteremia) (Falagas et al. 2007). Of interest, four of these isolates were MRSA and were recovered from patients who previously received vancomycin while three were VRE organisms, which are known to be less susceptible to daptomycin than *S. aureus* organisms, but for which recommended regimens are similar.

Optimizing Patients Outcomes by Optimizing Daptomycin Use

To improve patient outcomes and reduce the risk of emergence of resistance, it is essential to optimize the daptomycin dose and dosing interval. Multiple daptomycin safety/efficacy and PK studies have been conducted in different patient populations in order to establish its optimal dosing regimen.

Daptomycin is recommended to be administrated once daily. This regimen contributes to reducing potential toxicity without affecting efficacy, but also to facilitate adherence and significantly reduce the cost of therapy (Oleson et al. 2000; Safdar et al. 2004). In a recent study, Chakraborty et al. reported that both infusion over 30 min and injection over 2 min resulted in similar safety, tolerability, and exposure as judged by the observed AUC and peak concentrations (Chakraborty et al. 2009). This type of administration would be of particular interest in the management of patients with severe infections, making the dosing schedule easier, as well as reducing the volume of fluid administrated and the need for computerized pump administration.

High-Dose Daptomycin: Safety and Efficacy

The use of high-dose daptomycin therapy (up to 12 mg/kg/day for 14 days) has been reported to be well tolerated in healthy volunteers, with no serious adverse events, discontinuation of the therapy, or evidence of skeletal muscle toxicity (Benvenuto et al. 2006). Recent retrospective studies confirm these results in patients with Gram-positive infections and highlighted the significant improvement in patients' outcomes by using high-dose daptomycin (up to 15 mg/kg/day) without increasing the incidence of adverse events (Figueroa et al. 2009; McGee et al. 2009; Kullar et al. 2010). Bhavani et al. retrospectively investigated the relationships between increasing daptomycin dose and CPK elevation from a phase III evaluation of daptomycin for the treatment of *S. aureus* bacteremia and right-sided infective endocarditis trial. Of the 241 patients evaluated, 6 patients were found to have elevated CPK following daptomycin exposure of 6 mg/kg/day. They reported that *C* (min) values and CPK levels in these six patients were significantly correlated with *C* (min) of 24.3 mg/L or greater associated with increased likelihood of CPK toxicity (Bhavnani et al. 2010). Although higher doses of daptomycin (>8 mg/kg/day) are not approved by the FDA at this time, this strategy should be considered in future studies especially to maximize outcomes of patients with *S. aureus* and *Enterococci*-related infections and to reduce the risk of emergence of resistance.

Other Strategies

Antimicrobial deescalation and combination therapy are PK/PD-related strategies employed to optimize antimicrobial therapy. Antibiotic deescalation and escalation seem to be routinely employed by clinicians while attempting to reduce the risk of toxicity, cost of therapy, or to minimize the development of antibiotic resistance. These strategies imply changes in the dosing schedule of a drug given as monotherapy or switching a monotherapy regimen to bi- or tritherapy and vice versa. To our knowledge, there is little data available regarding microbiological outcomes secondary to deescalation and escalation of daptomycin therapy. Apart from clinical reports, there is only one in vitro PK/PD study investigating microbiological outcomes of daptomycin dose escalation and deescalation against MRSA and *h*VISA isolates over a period of 8 days. Results suggested that high-dose daptomycin early on would be beneficial in terms of PD activity against MRSA, hVISA, and VISA strains (Vidaillac et al. 2011). Further in vitro and in vivo studies including evaluation of a variety/wide range of isolates and increasing duration of exposure are warranted to better understand the potential benefit of such strategies.

Conclusion

Daptomycin represents a valuable antimicrobial for the treatment of Gram-positive infections. Its unique PK/PD properties and safety profile make daptomycin a preferred option for the treatment of bloodstream-associated and cSSSI infections. Although daptomycin therapy has been efficiently optimized since its early development, additional PK/PD studies further exploring its concentration-dependent cidal activity and surveillance studies documenting susceptibility pattern changes will have to be pursued to preserve the future utility of this antimicrobial agent.

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Chapter 16
PK/PD of Oxazolidinones

Ursula Theuretzbacher

Abstract Linezolid, the first available compound in the group of oxazolidinones, provides an effective alternative for the treatment of multidrug-resistant (MDR) Gram-positive bacteria. Linezolid's iv and oral availability expands its usage to the outpatient setting. In vitro, animal, and clinical studies have defined an appropriate PK/PD index for linezolid, its correlation with the dosage regimen, and clinical outcome. Due to linezolid's wide interpatient variability, some patients may have increased risk of inadequate drug exposure. As these patients are not readily identified, therapeutic drug monitoring may be necessary for critically ill patient populations as well as in long-term treatment. As alternative antibiotics are scarce, resistance development requires special attention. The selection of linezolid resistant mutants, especially with enterococci, and the emergence of mobile resistance determinants that affect a wide range of other ribosome-targeting antibiotics, will most likely spur the emergence and spread of linezolid resistance. Increasing drug exposure in an attempt to reduce selection pressure may not be feasible due to concentration dependent toxicity. On the other hand, combination therapy may positively impact exposure/resistance relationship, but our knowledge in this area remains incomplete. Employing PK/PD models to define dosing strategies and using antibiotic combinations to reduce selection pressure on linezolid-resistant mutants are major tasks yet to be undertaken.

Keywords Linezolid • Torezolid • Oxazolidinones • PK/PD • Pharmacodynamics • Pharmacokinetics • Resistance

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Introduction

Ribosomes serve as the site of mRNA translation and protein synthesis. The bacterial ribosome has a long history as a key target for antimicrobial compounds and presents multiple sites for antibiotic binding. Several classes of antibiotics function by binding to the bacterial ribosome and thus inhibiting bacterial protein synthesis, including the macrolides, ketolides, lincosamides, streptogramins, tetracyclines, chloramphenicol, aminoglycosides, pleuromutilins, as well as the synthetic oxazolidinones. Most of these drugs bind to only one subunit of the ribosome. In most instances, the binding involves specific sequences in the 16S or 23S rRNAs or additionally ribosomal proteins. The complex structure of the ribosome has been addressed by recent progress in structural, biochemical, and computational approaches, which has facilitated further exploitation of the ribosome as a drug target. As a result, potential interaction sites where new antibiotics can interfere with an essential ribosomal function have been illuminated (Polacek and Mankin 2005). Thus, designing and modifying drugs targeted at the highly validated bacterial ribosome has become a primary research goal for R&D companies in the anti-infective space.

After a pronounced gap in new antibacterial approvals, linezolid, the first member of the class of oxazolidinones, was introduced to the medical community in 2000 becoming the first antibacterial drug in more than 20 years to utilize a new mode of action. Though linezolid is the only currently available agent in this class, other oxazolidinones are in clinical development, one of them, tedizolid has been submitted to the U.S. Food and Drug Administration for approval. Development activities of these and other new analogs have spurred renewed interest in various aspects of PK/PD as a tool to predict the best dosage regimen; one that is effective while minimizing toxicity and reducing the risk of resistance development.

Linezolid's mode of action provides a treatment option against multidrug-resistant gram-positive pathogens without manifesting apparent cross-resistance to most other commonly used drug groups. Oxazolidinones target the ribosomal P site at a common antibiotic binding site on the ribosome, the ribosomal peptidyl transferase center (PTC) residing in the 50S ribosomal subunit. Oxazolidinones act by interfering with the formation of the complex that associates the mRNA and the initiator f-met-tRNA. The result is inhibition of the initiation step of bacterial translation (Long and Vester 2012; Leach et al. 2007; Wilson et al. 2008; Colca et al. 2003; Kalia et al. 2009; Aoki et al. 2002). However, the complexity of cross-resistance patterns between PTC binding antibiotics is likely due to the unique set of interactions that each bound antibiotic makes with the PTC cavity (Long and Vester 2012). Regarding the high degree of homology in the PTCs, it is not surprising that antibiotics that inhibit bacterial protein synthesis also inhibit mitochondrial protein synthesis (Tanel and Alexander 2006). In other words, with the structural similarity of the functionally critical regions of the mitochondrial and bacterial rRNA, some antibiotics also bind to the large subunit of mammalian mitochondrial ribosomes and thus inhibit mitochondrial translation (Bottger et al. 2001). This inhibition of mitochondrial protein synthesis is the likely cause of oxazolidinone's adverse effect of

myelosuppression (Nagiec et al. 2005; Kuter and Tillotson 2001; Duewelhenke et al. 2007). Indeed, oxazolidinones that are highly potent as antibiotics are also uniformly potent in inhibiting mitochondrial protein synthesis. Therefore, when developing new oxazolidinones, it will be crucial to evaluate potential mitochondrial toxicity (McKee et al. 2006). Linezolid has been targeted to several infections caused by Gram-positive bacteria. In the U.S., approved indications include vancomycin-resistant Enterococcus (VRE) faecium infections, nosocomial pneumonia caused by *Staphylococcus aureus* (methicillin-susceptible and -resistant strains—MSSA and MRSA), or *Streptococcus pneumoniae* (including multidrug-resistant strains), complicated skin and skin structure infections, including diabetic foot infections, without concomitant osteomyelitis, caused by *Staphylococcus aureus* (MSSA and MRSA), *Streptococcus pyogenes*, or *Streptococcus agalactiae*, uncomplicated skin and skin structure infections caused by *Staphylococcus aureus* (MSSA only) or *Streptococcus pyogenes*, and community-acquired pneumonia (CAP) caused by *Streptococcus pneumoniae* (including multidrug-resistant strains, including cases with concurrent bacteremia, or *Staphylococcus aureu*s (MSSA only). In Europe, the approved indications are nosocomial pneumonia, community-acquired pneumonia, and complicated skin and soft tissue infections according to the microbiological test result.

Pharmacokinetic/pharmacodynamic (PK/PD) concepts link exposure patterns with effects (Ambrose PaulÂ et al. 2007; Drusano 2007). The derived PK/PD index is correlated with a measure of clinical or microbiological outcome to obtain a cut-off target value that predicts outcome. This value is then used to optimize dosage regimes. All PK/PD indices have a component of drug concentration (e.g. peak concentration, AUC) and a PD component (MIC). In the clinical situation, the PD component (MIC) is an observed reality, while the PK component may be manipulated by dosing and individually adapted. The goals of an optimized dosing regimen should include maximizing antimicrobial activity, minimizing the probability of a toxic effect, and reducing the selection pressure on resistant subpopulations.

Pharmacokinetics of Linezolid

This book chapter focuses on PK/PD of oxazolidinones. Therefore, mainly PK parameters that are directly relevant for PK/PD issues are discussed in the following paragraphs.

Linezolid's pharmacokinetic profile has been studied in healthy volunteers as well as in various patient groups. Due to its almost 100 % bioavailability, iv and oral exposure data can be exchanged with no need to be considered separately. Linezolid has a relatively low protein binding of about 10–30 % (Dehghanyar et al. 2005; MacGowan 2003; Thallinger et al. 2008). In critically ill patients, the percentage bound has a large variation depending on serum albumin levels (Yagi et al. 2013). Though relatively low, protein binding should not be overlooked, especially when relating total (bound + free) concentrations to routine MICs measured in protein-free media. In general, only free concentrations should be used in PK/PD studies. However, in most published studies linezolid concentrations are given as total

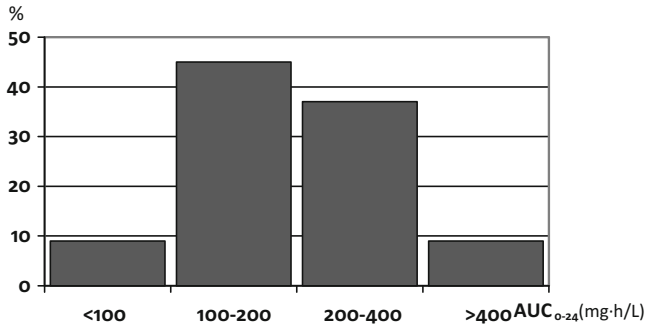

Fig. 16.1 Distribution of $AUC_{0–24}$ values (mg h/L) in patients under the linezolid compassionate-use program (Meagher et al. 2003), reproduced with permission

concentrations and, in these cases the available active drug concentrations may be slightly overestimated.

PK/PD parameters, such as AUC/MIC, C_{max}/MIC, or $t>$MIC, have been related to clinical and bacteriological efficacy and to emergence of resistance as well as to toxicity. The pharmacokinetic parameters AUC, C_{max}, C_{min}, and concentrations at various infection sites are primary factors in determining PK/PD relationships. These factors will be the main focus in the next paragraphs as they discuss selected PK characteristics of linezolid. Most of these studies have used a dosage of linezolid 600 mg q12h as this exact dosage is approved in all countries.

Mean $AUC_{0–24}$ values in patients at steady state at a standard dosage of 600 mg q12h ranges from 150 to 260 mg h/L (Smith et al. 2003; Adembri et al. 2008; Meagher et al. 2003) with high interpatient variability (60–870 mg h/L) (Fig. 16.1) (Meagher et al. 2003). The mean C_{max} is approximately 14 µg/mL (Whitehouse et al. 2005), and C_{min} approximately 1–6 µg/mL (Adembri et al. 2008; Whitehouse et al. 2005). Table 16.1 shows pharmacokinetic parameters after single or multiple dosing in volunteers and patients. Multiple-dose studies show a limited but significant dose-dependent accumulation (Fig. 16.2) (Burkhardt et al. 2002). Accordingly, for PK/PD analysis, PK parameters at steady state should be used.

In healthy adult persons, the reported mean elimination half-life has ranges from 4 to 7 h in both single-dose and steady-state conditions (Bosso et al. 2004). Clearance occurs by both renal and nonrenal (65 %) mechanisms (Brier et al. 2003). With both oral and iv administration, there is a wide variability in clearance that can be accounted for primarily by variability in nonrenal clearance (Stalker et al. 2003). A recent study observed a remarkable decrease in linezolid clearance (approximately 50 % decrease) in patients with severe liver cirrhosis (Child Pugh grade C), suggesting that cirrhosis changes the pharmacokinetics of linezolid (Sasaki et al. 2011).

About 30–40 % of the dose is recovered in urine as parent compound within the first 12–120 h (Burkhardt et al. 2002; Wagenlehner et al. 2003). Recent studies with a limited number of patients showed the relationship of renal function to linezolid clearance, with subsequent accumulation, and thus, potential toxic events (Matsumoto et al. 2010, Yagi et al. 2013). Interestingly, the nonrenal clearance may

Table 16.1 Mean pharmacokinetic parameters of linezolid (total drug)

Dosage	Population (*n*)	AUC_{0-24} (mg h/L)	C_{max} (μg/mL)	C_{min} (μg/mL)	Vd	Total clearance	Elimin. half-life (h)	Reference
600 mg iv, SD (normalized from 625 mg)	Adults volunteers	$AUC_{0-\infty}$: 80.20 ± 33.3	12.9 (SD1.6)			138 ±39 mL/min	4.4 ±2.4	FDA (2010)
600 mg iv, MD (normalized from 625 mg)	Adult volunteers	$AUC_{0-\infty}$: 89.7 ± 31	15.1 ± 2.5			123 ± 40 mL/min	4.8 ± 1.7	FDA (2010)
375 mg iv, SD	Adult volunteers (*n* = 12)		10.3 ± 1.9 mg/L		42.3 ± 6.7 L	7.3 ± 2.0 L/h	4.4	Sisson et al. (1999)
600 mg oral, SD	Adult volunteers (*n* = 6)	$AUC_{0-\infty}$: 110 ± 22	12.7 ± 2.6			94.6 ± 21.8 mL/min	6.4 ± 2.2	Brier et al. (2003)
625 mg oral, MD	Adult volunteers (*n* = 24, oral, *n* = 18, iv)	AUC_{0-12}: 147 ±58 oral, 93.4 ± 32.3 iv	18.8 ± 6.2 oral, 15.7 ± 2.6 iv	8.02 (3.6) oral, 3.8 (2.5) iv	36.1 (10.5) oral, 45.5 (4.9) iv	78.2 ±23.3 mL/min oral, 123 ± 40.3 iv	5.4 ± 0.9 oral, 4.8 ± 1.7 iv	Stalker et al. (2003)
600 mg iv, MD	Critically ill patients (*n* = 28)		14.0 ± 4.5	12 h: 1.4 ± 1.1;24 h: 2.8 ± 2.7; 48 h: 3.9 ± 4.4; 72 h: 4.5 ± 6.2	41.2 L/65 kg	0.049 ± 0.016 L/h/kg, 20–179 mL/min without, 31–91 mL/min with renal support		Whitehouse et al. (2005)
600 mg iv/oral, MD	Patients (*n* = 318), MD	228 (CV 50.3 %, range 57–871)			1 L/kg (CV 23 %)	0.1 L/h/kg (CV 50 %)		Meagher et al. (2003)

(continued)

Table 16.1 (continued)

Dosage	Population (*n*)	AUC_{0-24} (mg h/L)	C_{max} (μg/mL)	C_{min} (μg/mL)	Vd	Total clearance	Elimin. half-life (h)	Reference
600 mg iv, MD	Neutropenic patients (*n* = 56)	212 (CV 49 %)			0.95 L/kg	0.13 L/h/kg		Smith et al. (2003)
600 mg iv, MD	ICU patients (*n* = 9)	154.2 ± 59.6	13.1 ± 4.3	1.1 ± 1.5				Adembri et al. (2008)
600 mg iv, SD	Patients with sepsis or septic shock (*n* = 24)	$fAUC_{0-24}$: 70.8 ± 28.1 (septic shock), 50.9 ± 23.1 (severe sepsis)	fC_{max}: 14.2 ± 3.5 (septic shock), 14.2 ± 4.1 (severe sepsis)		60.4 ± 13.9 L (septic shock), 57.2 ± 17.8 L (severe sepsis)	9.8 ± 4.3 L/h (septic shock), 14.8 ± 7.5 L/h (severe sepsis)	4.9 ± 2.1 (septic shock), 3.1 ± 1.5 (severe sepsis)	Thallinger et al. (2008)
600 mg iv, SD	CF adult patients (*n* = 12)	$AUC_{0-\infty}$: 112.2 ± 46.4			0.87 (±0.2) L/kg	0.12 ±0.06 L/h/kg	4.4 ± 2.4 (range 1.8–8.4)	Bosso et al. (2004)
10 mg/kg, SD	Infants (>28 days to <3 months, *n* = 12)	$AUC_{0-\infty}$: 33 (CV 26 %)	11 (CV 27 %)		0.8 (CV 26 %) L/kg		5.4 (CV 32 %)	FDA (2010)
10 mg/kg, SD	Children (3 months to 11 years, *n* = 59)	$AUC_{0-\infty}$: 58 (CV 54 %)	15.1 (CV 30 %)		0.7 (CV 28 %) L/kg	0.34 ± 0.15 L/h/kg	3.8 (CV 53 %)	FDA (2010)
10 mg/kg iv q 8 h, MD	CF pediatric patients (*n* = 10)		Range 8.4–20.5	Range 0.1–11.5				Santos et al. (2009)

SD single dose, *MD* multiple doses

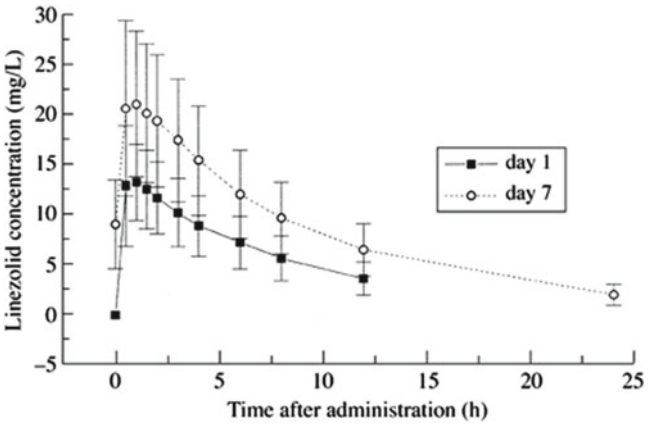

Fig. 16.2 Mean (S.D.) total serum concentrations of linezolid in healthy volunteers on day 1 and day 7 (Burkhardt et al. 2002), reproduced with permission

be saturated by reduced renal clearance (Meagher et al. 2003), which potentially explains the discrepancy in the contribution of renal function to linezolid clearance in many studies. Additionally, it has been suggested that linezolid reduces its own metabolism via inhibition of the mitochondrial respiratory chain enzyme activity with resulting nonlinearity (Plock et al. 2007).

Linezolid is metabolized by an oxidative reaction to two inactive metabolites, a hydroxyethyl glycine metabolite (40 % of the dose in urine) and an aminoethoxyacetic acid metabolite (10 % of the dose in urine) (Brier et al. 2003; Kazuaki et al. 2010; Slatter et al. 2001). These two major metabolites have been found to accumulate in patients with renal failure (Brier et al. 2003), but their toxicity profile has not been well described. Thus, linezolid is not a typical renal excretion-type drug and linezolid is not metabolized by the P-450 enzyme system (Wynalda et al. 2000). Pharmacokinetic data for linezolid are summarized in Table 16.1.

Comparing serum concentrations adjusted to 70 kg body weight, the distribution volume is significantly lower and concentrations higher in females than in males (Burkhardt et al. 2002). This effect is not only observed in healthy adults but also in patients.

In clinical practice the substantial interpatient variability is remarkable (Fig. 16.3) and might be responsible not only for a reduced exposure and risk for both suboptimal activity and for resistance development but also for increasing the risk of toxicities. The wide range of reported half-lives and clearance values in healthy adults and patients also, at least partly, is caused by nonlinear elimination, which is probably a result of the saturability of one of the two major metabolic pathways for the drug (Bosso et al. 2004; Plock et al. 2007). This pronounced variability may have implications for proper dosing and thus adequate drug exposure in individual patients. Additionally, the drug exposure may change in an individual patient at different time points of drug therapy (Fig. 16.4). A recent retrospective observational study assessed the interindividual pharmacokinetic variability in daily clinical practice in

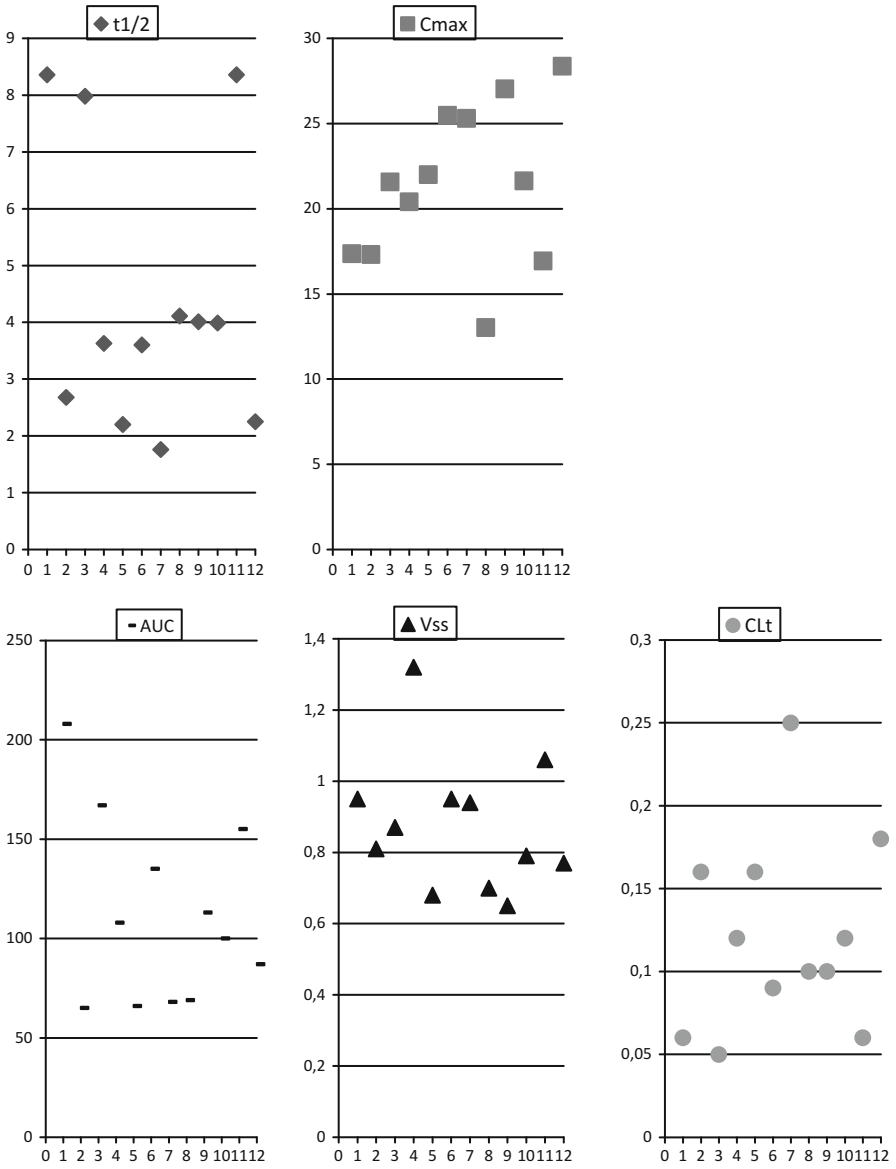

Fig. 16.3 Pharmacokinetic parameters of individual patients with CF (Bosso et al. 2004), reproduced with permission. $t_{1/2}$ (h) half-life, C_{max} (μg/mL) maximum observed concentration in plasma, $AUC_{0-\infty}$ (μg h/mL) area under the concentration in plasma versus the time curve from time 0 to infinity; V_{SS} (L/kg) steady-state volume of distribution, Cl_T (L/h/kg) total body clearance

medical, surgical, or intensive care units. In this limited study 30–40 % of patients were either under- or overdosed and therapeutic drug monitoring (TDM) was suggested (Pea et al. 2010)). Similar results were obtained in another study with

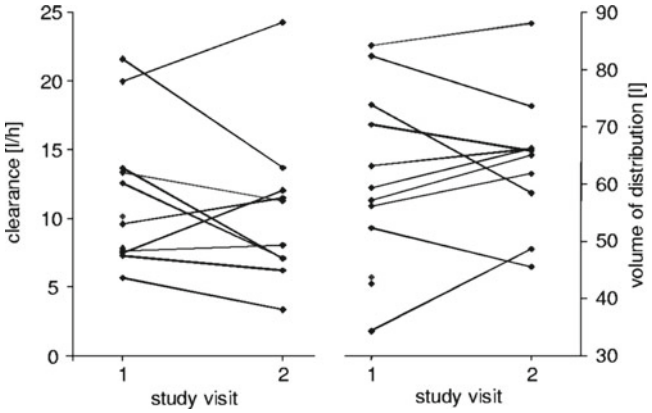

Fig. 16.4 Individual changes in clearance and volume of distribution of unbound linezolid in plasma after multiple iv dosing of 600 mg twice a day (each symbol represents the estimated parameter value for one patient) (Buerger et al. 2006), reproduced with permission

inadequate concentrations in 30% of patients, especially in those with glomerular filtration rate of > 80 ml/min and staphylococcal infections treated in the intensive care unit (Morata et al. 2013).

Linezolid Concentration at the Site of Infection

In most PK/PD analyses, the pharmacokinetics generally used are those in the blood (plasma or serum) because these are easily accessible and available. However, this approach is incongruent with the reality that most infections occur, not in the plasma, but rather in tissue sites (Theuretzbacher 2007). It is well accepted that the ability of antibiotics to reach target sites is a key determinant of clinical outcome. With few exceptions, such as infections caused by intracellular bacteria, the interstitial fluid (IF) of tissue and other body fluids represent the actual target space for the preponderance of bacterial infections. If the site of infection is the IF or other body fluid without significant barriers and without influx or efflux mechanisms, rapid equilibrium between the tissue fluid and plasma can be expected. Depending on the surface area-to-volume ratio, the free plasma concentration would be equal to the IF and could serve as a surrogate marker for IF concentrations (Ryan et al. 1986; April et al. 2010).

Linezolid belongs to the group of drugs with a volume of distribution that approximates the total body water content (40–50 L), i.e., linezolid concentrations in interstitial fluid are very close to those of plasma (Stalker et al. 2003). Additionally, to a certain extent linezolid penetrates into human cells according to its volume of distribution. In accordance with the results of linezolid's pharmacokinetics in blood, variability in tissue penetration across studies in healthy volunteers and patients is seen (Stein and Wells 2010).

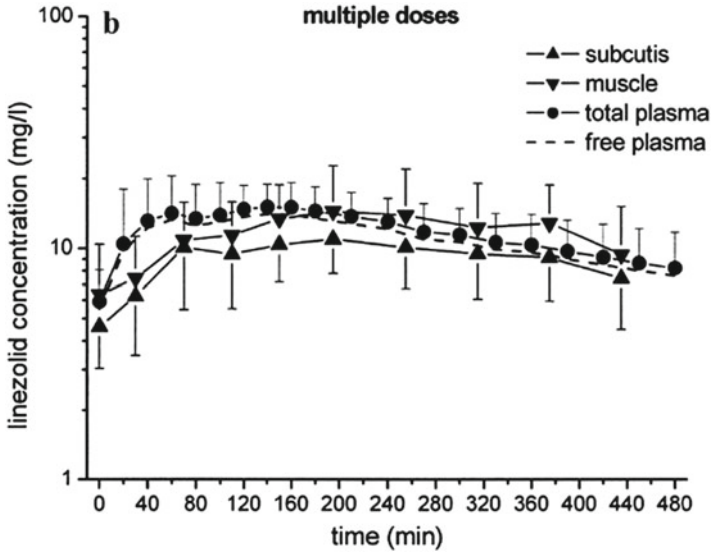

Fig. 16.5 Time–concentration curves (means ± SDs) of linezolid in plasma (total and free) and IF of subcutaneous adipose tissue and skeletal muscle after administration of multiple doses of oral linezolid intake (600 mg twice a day; $n = 9$) (Dehghanyar et al. 2005), reproduced with permission

Regardless of Linezolid's low protein binding of about 30 %, it is still preferable to determine free concentrations, not only in plasma but also in other compartments, especially if these values are used as PK input for PK/PD analysis. Linezolid's protein binding is not concentration dependent but is quite variable between and within patients (Wiskirchen et al. 2011). Therefore, in vivo microdialysis methods that measure only free concentrations in body fluids over time provide valuable data. In healthy volunteers, the unbound plasma fraction of linezolid equilibrates completely with the IF concentrations in adipose tissue and skeletal muscle (Fig. 16.5) (Dehghanyar et al. 2005).

Linezolid is approved for treatment of lung infections. Epithelial lining fluid (ELF) is widely accepted to be representative of the environment in which extracellular pulmonary pathogens are most often located. Although determination of concentrations in the ELF is challenging due to various technical issues and potential sources of error, ELF concentration data may be useful as PK input in PK/PD analysis. Boselli et al. determined the steady-state plasma pharmacokinetic variables and ELF concentrations of standard dosage of linezolid administered to critically ill patients with ventilator-associated pneumonia. The mean ELF peak and trough concentrations of 14.4 ± 5.6 μg/mL and 2.6 ± 1.7 μg/mL (Boselli et al. 2005), respectively, shows a mean linezolid percentage penetration in ELF of approximately 100 %. This penetration ratio has been confirmed in a study with continuous infusion (Boselli et al. 2012). These results are indicative of passive diffusion of the unbound drug across membranes (Ryan et al. 1986). Other results from studies that found higher linezolid concentrations in ELF may have been caused by technical errors in the process of bronchoalveolar lavage (Kiem and Schentag 2008).

As expected from linezolid's distribution in the total body water, the concentrations in alveolar macrophages are rather low (Conte et al. 2002; Honeybourne et al. 2003).

Other body fluids and their linezolid concentrations have been investigated. Mean concentrations of linezolid in the hematoma fluid drained from around the surgical site were 8.2 μg/mL at 6–8 h and 5.6 μg/mL at 10–12 h after the infusion, and 7.0 μg/mL at 2–4 h following a second 600 mg infusion given 12 h postoperatively. This indicates therapeutic concentrations at the operation site of >16 h (Lovering et al. 2002). Stein et al. determined the pharmacokinetics of patients with peripheral vascular disease and severe diabetic foot infections requiring surgical intervention. Their results showed lower than expected IF concentrations in tissues that was explained by impaired blood flow in this small study population (Stein et al. 2007).

Linezolid concentrations have been studied in ventricular fluid of hydrocephalic children and adolescents with linezolid administration of 10 mg/kg every 12 h with C_{max} values of 7.5 μg/mL (range 2.3–12.6 μg/mL) and C_{min} values of 1.3 μg/mL (range 0.2–2.6 μg/mL). The study confirmed the high interpatient variability found in other investigations (Yogev et al. 2010). In adults, linezolid penetrated into the cerebrospinal fluid with C_{max} and C_{min} concentrations during a standard dosage regimen of 10.8 ± 5.7 μg/mL and 6.1 ± 4.2 μg/mL, respectively (Myrianthefs et al. 2006).

Pharmacokinetic Interactions

Linezolid is not an inducer of the cytochrome P450 system in rats and does not inhibit the activities of clinically significant human CYP isoforms (e.g., 1A2, 2C9, 2C19, 2D6, 2E1, and 3A4) (FDA 2011a); however, the mechanism of interaction via hepatic enzymes is not well understood. The clinically relevant interactions that have been reported to the U.S. Food and Drug Administration (FDA) Adverse Event Reporting System (FDA 2012) most commonly involved warfarin, statins, serotonin antagonists, clarithromycin, and varenicline. As linezolid is a reversible, nonselective inhibitor of monoamine oxidase, it has the potential for interaction with adrenergic and serotonergic agents as noted in the FDA reporting system (FDA 2012; Lawrence et al. 2006). The FDA has also received reports of serious central nervous system reactions when linezolid is administered to patients taking serotonergic psychiatric medications (FDA 2011b). Accordingly linezolid should generally not be given to patients taking serotonergic drugs (FDA 2011b). Additionally, due to potential interactions with adrenergic agents, patients taking linezolid should avoid consuming large amounts of foods or beverages that have high tyramine content.

Even though not an inducer of the cytochrome P450 system, linezolid is affected by P-glycoprotein inducers such as rifampicin (Gebhart et al. 2007). Rifampicin induces P-glycoprotein that enhances the nonrenal clearance of linezolid, thus reducing linezolid exposure (Egle et al. 2005). This effect may explain the preventive effect of rifampicin on bone marrow toxicity in patients receiving linezolid/rifampicin combination therapy (Pea et al. 2012). However, this beneficial effect of rifampicin combination therapy could theoretically lead to therapeutic failure in

some patients and could favor the spread of resistant strains. TDM is recommended to treat such patients effectively. Additionally, other P-glycoprotein substrates and inhibitors, such as proton pump inhibitors, may cause clinically significant interactions with linezolid (Pea et al. 2012).

Pharmacokinetics in Special Patient Groups

Pediatric Patients

Children younger than 12 years of age have a smaller AUC, a faster clearance and a shorter elimination half-life than adults after administration of linezolid. Although clearance rates in newborn infants are similar to those in adults, clearance increases rapidly during the first week of life, becoming two- to threefold higher than in adults by the seventh day of life. The clearance of linezolid decreases gradually among young children, becoming similar to adult values by adolescence. In children age 12 years and older, the pharmacokinetics of linezolid is not significantly different from that of adults. Because of the higher clearance and lower AUC, a shorter dosing interval for linezolid is required for children younger than 12 years of age in order to produce adequate drug exposure (Jungbluth et al. 2003; Kearns et al. 2000).

Patients with Impaired Renal or Hepatic Function

The renal clearance of linezolid is low (average 40 mL/min). Therefore, renal insufficiency is generally regarded as not altering the pharmacokinetics of the parent drug linezolid, whereas its metabolites do accumulate (Meyer et al. 2005). According to recent study results, renal insufficiency influences the clearance of linezolid and, so, TDM is recommended for selected severely ill patients (s. page). As linezolid is partly removed by hemodialysis (in 3 h approximately 30 %), serum linezolid levels in critically ill patients with acute renal failure and renal replacement therapy can be significantly reduced (Brier et al. 2003; Fiaccadori et al. 2004, 2006). The best method of managing linezolid dosage in these complex patient groups, would be to use TDM (Swoboda et al. 2010). Similar to the high variability in nonrenal clearance (Stalker et al. 2003), patients with liver transplantation or resection have a substantially reduced linezolid clearance with high interindividual variability (Swoboda et al. 2010). The pattern of drug exposure in patients with insufficient renal function (CLcr $\leq$ 30 mL/min) or severe liver cirrhosis (Child-Pugh grade C) is mirrored by a remarkably increased risk for thrombocytopenia (Sasaki et al. 2011; Forrest et al. 2000). Indeed, in patients with severe renal insufficiency (CLcr $\leq$ 10 mL/min) or severe liver cirrhosis, high incidences of thrombocytopenia were estimated even at 600 mg/day due to elevated exposure levels and low baseline platelet counts. Hence, careful monitoring of platelet counts is strongly advised for these patients (Sasaki et al. 2011).

Obese Patients

Limited data on linezolid dosing in the morbidly obese population show lower serum drug concentrations than those in nonobese patients with potential treatment failure (Muzevich et al. 2013). Two studies have shown increased clearance of linezolid and reduced serum concentration compared to population pharmacokinetic parameters, with trough levels below MIC_{90} (Tsuji et al. 2012a; Stein et al. 2005). Linezolid undergoes slow nonenzymatic oxidation in vivo that may be increased in obese patients, and this may account for the greater clearance (Tsuji et al. 2012a). Another study in morbidly obese adults found a significant positive relationship for the total volume of distribution with total body weight, adjusted body weight, lean body weight, and ideal body weight, but not with body mass index (Bhaldodi et al. 2013).

Critically Ill Patients

In critically ill patients various pathophysiological changes often impact pharmacokinetics of antimicrobials. In this patient population, linezolid clinical studies have observed considerable differences in mean PK parameters, such as Vd and clearance which may influence outcome. Increased Vd (as a result of edema in sepsis and trauma, pleural effusion, ascites, mediastinitis, fluid therapy, or indwelling postsurgical drainage) and/or enhanced renal clearance (as a result of burns, drug abuse, hyperdynamic conditions during sepsis, acute leukemia, or use of hemodynamically active drugs) may cause underexposure. On the other hand, overexposure may occur because of a drop in renal clearance caused by renal impairment (Pea et al. 2005).

Pharmacokinetics of linezolid has been evaluated in a wide range of critically ill patients. In a study with patients with major thermal injuries, an AUC reduced by half, compared with volunteers, has been described. This is primarily attributed to increased nonrenal clearance. For patients with very extensive or complicated burns, where interpatient variability is large, insufficient drug exposure may occur (Lovering et al. 2009). Other clinical studies with critically ill patients confirm the increased volume of distribution and clearance (Meagher et al. 2003; Buerger et al. 2006; Boselli et al. 2005). The higher volume of distribution in these patients might be caused by fluid retention and/or high fluid input resulting in significant changes in surface area-to-volume ratio of infection sites. Increased oxidative stress in septic patients, compared to noncritically ill patients, may cause the more rapid elimination as linezolid is metabolized by nonenzymatic oxidation (Meagher et al. 2003). The changed PK parameters may result in lower AUC values with an extremely broad range of more than ten times difference and with the resulting potential for inadequate drug exposure in individual patients (Meagher et al. 2003).

Buerger et al. studied free linezolid concentrations in plasma and IF of subcutaneous adipose tissue and skeletal muscle of critically ill patients (Buerger et al. 2006). Though median plasma concentrations were satisfactory, the concentrations in IF of some patients remained below the MIC for a large proportion of or, even, the entire dosing interval (Buerger et al. 2006). This variability again underlines the

Table 16.2 Mean pharmacokinetic parameters for targeted patient populations (Meagher et al. 2003)

Parameter	Value				
	ICU patients[a] ($n=94$)	Obese patients[b] ($n=95$)	Oral linezolid therapy[c] ($n=87$)	Elderly patients ($n=74$)[d]	All patients ($n=318$)
V_c (L/65 kg)	39.8 (25)	43.9 (18)	39.3 (19)	38.2 (21)	39.6 (23)
V_{ss} (L/65 kg)	67.7 (24)	69.7 (18)	65.1 (25)	64.2 (22)	65.8 (23)
CL_{ratio}	0.288 (32)	0.298 (28)	0.247 (32)	0.269 (32)	0.269 (34)
K_m (µg/mL)	1.38 (42)	1.53 (62)	1.45 (64)	1.53 (56)	1.46 (68)
CL_i (L/65 kg)	46.8 (59)	43.8 (45)	40.7 (41)	40.7 (37)	43.5 (53)
V_{max} (mg/h/65 kg)	55.8 (28)	57.4 (26)	49.2 (20)	53.8 (25)	53.3 (26)
AUC (µg/mL 24 h)	206 (60)	210 (56)	258 (56)	269 (54)	228 (58)
CL_{tavg} (L/h/65 kg)	7.65 (50)	7.27 (49)	5.86 (46)	5.68 (52)	6.85 (50)

[a]Patients may be represented in more than one category. The CV (percent) is shown in parentheses
[b]Patients were categorized as obese if total body weight was >30 % above the calculated IBW
[c]Patients were either started on oral linezolid or switched to oral therapy following initiation of iv linezolid
[d]Patients >70 years of age were considered to be elderly

possibility of decreased activity or promotion of resistance development in patients with low linezolid concentrations. The high interindividual differences that were seen even with healthy volunteers are consistently mirrored in all studies with patients (Table 16.2) (Dehghanyar et al. 2005; Buerger et al. 2006).

Pharmacokinetic data from neutropenic patients are only reported in the literature, it from a compassionate use program and showed comparable serum values across all severely ill patients (Smith et al. 2003). It may be interesting to note that results from the neutropenic mouse model showed a marked reduction in pulmonary drug exposure compared to immunocompetent mice. The reasons for this discrepancy are not evident (Keel et al. 2012a).

Pharmacodynamics

Antimicrobial Activity

Pharmacodynamics defines the exposure–response relationship. It is routinely measured as MIC, a measurement at a fixed concentration at a single point in time. To obtain information about bacterial growth and kill over time, static or dynamic time–kill curves are used.

Linezolid is active against many Gram-positive organisms but is not able to reach its intracellular site of action in Gram-negative bacteria due to effective RND-type efflux pumps (Schumacher et al. 2007). Common Gram-positive bacteria resistant to other antibiotics, including MRSA, penicillin-resistant *S. pneumoniae,* and VRE are susceptible to linezolid.

The in vitro activity against *S. aureus* is very uniform and the wild-type distribution narrow. About 90 % of clinical strains in Europe ($n \geq 62{,}000$) have an MIC of

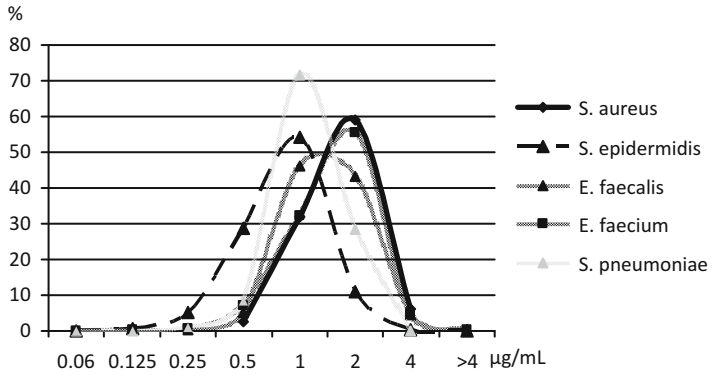

Fig. 16.6 Wild-type MIC distribution of Gram-positive bacteria to linezolid (EUCAST strain collection)

1 µg/mL (32 %) or 2 µg/mL (59 %) according to the EUCAST strain collection (Rationale Documents—Linezolid 2005). MRSA and MSSA are equally inhibited by linezolid. The majority of *S. epidermidis* MICs are shifted one dilution step to the left but with the same MIC90 value of 2 µg/mL (EUCAST wild-type distribution) (Fig. 16.6). A global surveillance program found that MIC values have not changed over the years since the introduction of linezolid (Farrell et al. 2009). Enterococci show MIC_{50} and MIC_{90} values at 1 and 2 µg/mL, respectively (Farrell et al. 2009). Enterococci are inhibited independent of their resistance to vancomycin. As the MIC distribution curve extends to an MIC of 4 in Gram-positive cocci, the epidemiological cutoff value has been set at an MIC of 4 µg/mL for enterococci and *S. aureus*, according to the EUCAST rational document (EUCAST Steering Committee 2006). The PK/PD based breakpoint would be considerably lower at <1 µg/mL.

The action of linezolid is considered to be bacteriostatic against staphylococci and enterococci (Fig. 16.7) (Rybak et al. 2000). In vivo experiments show that, according to this activity pattern, the bacterial load decreases only gradually after administration of linezolid as the phagocytes eliminate the bacteria (Miyazaki et al. 2002). Increasing doses produces minimal concentration-dependent killing (Andes et al. 2002). As linezolid does not decrease bacterial density effectively, it may not be successful in patients with complicated sequestered infections such as infective endocarditis. Indeed, in vitro and animal studies as well as clinical studies have been shown inferior activity and treatment failures in endocarditis (Chiang and Climo 2003; Ruiz et al. 2002). Linezolid shows a considerable off-label usage in febrile neutropenic patients. It is still not clear if a bacteriostatic drug like linezolid is appropriate for the treatment of life-threatening infections in neutropenic patients if, alternatively, bactericidal agents are available for treatment (Theuretzbacher 2012).

As some infections, such as pneumonia or endocarditis, can yield a high number of bacteria at the site of infection, the impact of a high inoculum has been evaluated. In contrast to vancomycin, linezolid is not affected by a high inoculum in a published in vitro experiment (LaPlante and Rybak 2004). However, the required dose for a static effect against S. aureus increased 4-fold at 107 CFU in the mouse thigh model (Lee et al. 2013).

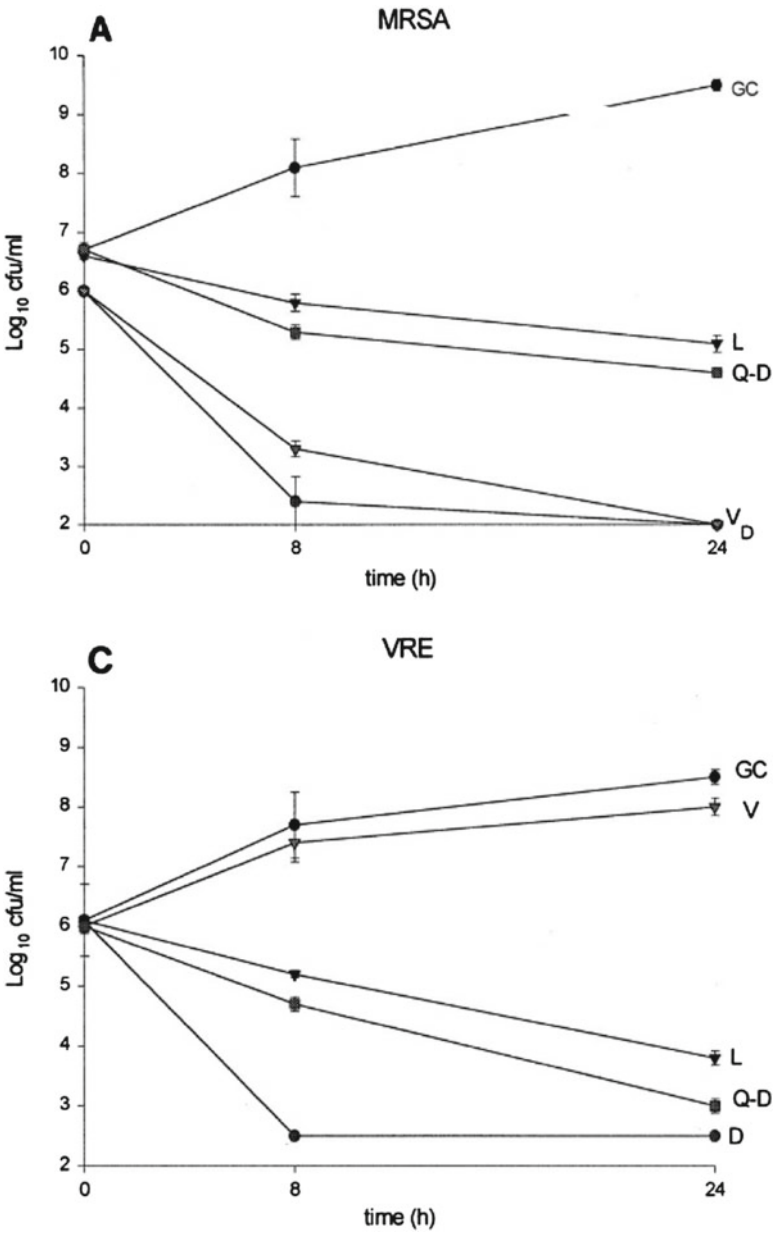

Fig. 16.7 Time–kill experiments performed at four times the MIC against MRSA R499* (A) and VRE R588* (*E. faecalis*) (C) (Rybak et al. 2000), reproduced with permission. Results are means ± standard deviations. *GC* growth control, *D* daptomycin, *V* vancomycin, *L* linezolid, *Q–D* quinupristin–dalfopristin. *MICs of D, V, L, and Q–D: for MRSA 0.125, 0.5, 2.0, and 0.25; for VRE 1.0, 128.0, 4.0, and 2.0

As *Staphylococcus aureus* is able to survive in phagocytes, the intracellular activity of linezolid was studied. In vitro, linezolid showed an E_{max} of ~1 $\log_{10}$ cfu reduction compared with initial inoculum both intra- and extracellularly. In vivo, the efficacy of linezolid was impaired and failed to reduce the cfu to less than the initial load intracellularly (Sandberg et al. 2010; Lemaire et al. 2009). Linezolid exerts only a weak intracellular activity against the strains of *S. aureus* tested and remains considerably less active than other bactericidal antistaphylococcal drugs (Sandberg et al. 2010).

Linezolid is predominantly bacteriostatic with some persistent antibiotic effects. Though controversy remains, an in vivo postantibiotic effect (PAE) may influence the effectiveness of dosage regimes. Linezolid exhibits moderate concentration-dependent in vitro PAE against *S. aureus*, *S. epidermidis*, *E. faecalis*, *E. faecium,* and *S. pneumoniae* (Munckhof et al. 2001) with ranges from 0.8 to 3 h in vitro and 3 to 4 h in vivo (Andes et al. 1998; Rybak et al. 1998). No in vivo PAE was found with *S. pneumonia* (Andes et al. 2002).

Resistance

Resistance is a major concern in treating severely ill patients with infectious diseases. In general, resistance rates to linezolid remain very low as is to be expected due to its short period of availability of only 13 years (Flamm et al. 2013). Nevertheless, oxazolidinone-resistant strains have appeared worldwide particularly among enterococci and coagulase-negative staphylococci (Jones et al. 2009a; Farrell et al. 2011a; Gu et al. 2013). In a concerning trend, increasing linezolid utilization rates and clonal spread have led to outbreaks of both linezolid-resistant enterococci and linezolid-resistant MRSA (Mulanovich et al. 2010; Sanchez Garcia et al. 2013).

In general, target site mutations are one of the most common resistance mechanisms. In the case of linezolid, resistance is usually associated with a point mutation at the drug target site at position 2576 of 23S rRNA. However, other mutations have been found in clinical isolates (Lovering et al. 2009) or selected for in serial passage experiments (Locke et al. 2009). Most bacteria have several copies of rRNA genes and significant resistance requires mutations in more than one copy of the 23S rRNA gen. MICs increase in proportion to the number of copies of mutant 23S rRNA genes (Livermore et al. 2009). Therefore, the number of available alleles (*M. tuberculosis*>enterococci>*S. aureus*) determines the emergence of resistance as has been shown with treatment failures due to the emergence of linezolid-resistant mutants with enterococci (Ntokou et al. 2012a; Santayana et al. 2012) but only rarely with staphylococci (Ba et al. 2010). Serial passage experiments readily resulted in selection of linezolid-resistant mutants of *E. faecalis* (Fig. 16.8). These experiments predict the rapid emergence and spread of resistant *E. faecalis* strains within medical facilities (Prystowsky et al. 2001). Also, the threat of multiclonal outbreaks of enterococci in intensive care units is well known (Ntokou et al. 2012b; Gómez-Gil et al. 2009). Alarmingly, clinical isolates of linezolid-resistant *S. aureus* with all five

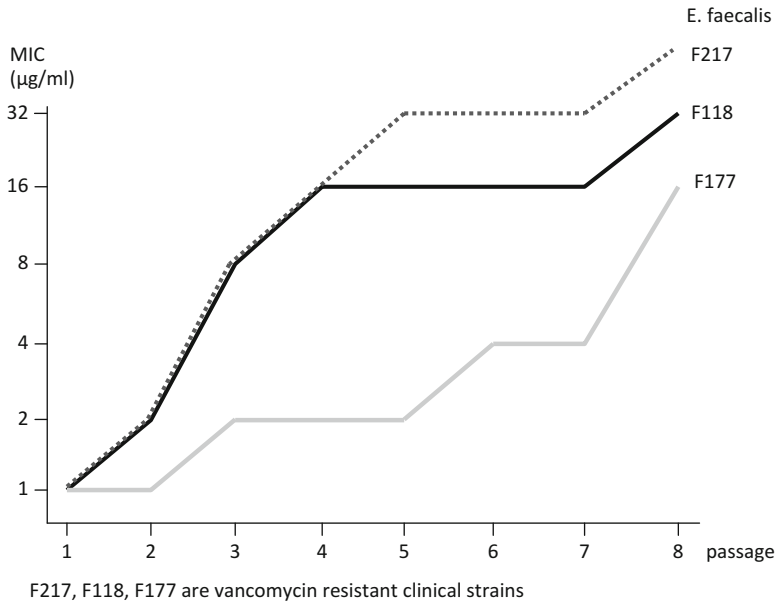

Fig. 16.8 In vitro selection of linezolid-resistant enterococci (Prystowsky et al. 2001), reproduced with permission; F217, F118, and F177 are vancomycin-resistant clinical strains

copies of the 23S rRNA gene containing the G2576U mutation have been found (Pillai et al. 2002). Additionally, a variety of mutations in ribosomal protein L3 and L4 have also been identified that confer reduced susceptibility to oxazolidinones and may cooccur with other resistance mechanisms (Locke et al. 2010). Other mechanisms of resistance to linezolid include a decrease in the antimicrobial uptake and more mechanisms may be discovered in the future (Sierra et al. 2009). Although the ribosomal proteins L3 and L4 are located farther away from those binding drug, mutations in specific regions of these proteins are increasingly being associated with linezolid resistance (Long and Vester 2012).

The recently described natural, mobile, and ribosomal-based resistance mechanism (*cfr* = chloramphenicol–florfenicol resistance) has emerged in *S. aureus* and *S. epidermidis*, especially in MRSA, E. faecalis E. faecium, and has now been documented in several continents and in outbreak settings (Farrell et al. 2009; Gao et al. 2010; Arias et al. 2008; Witte and Cuny 2011; Patel et al. 2013; Baos et al. 2013). These findings show that linezolid, as a synthetic compound, is not protected from natural resistances as has been postulated before. The product of the natural *cfr* gene is a methyltransferase that catalyzes methylation of A2503 in the 23S rRNA gene of the large ribosomal subunit. The generally low fitness cost of *cfr* acquisition explains the apparent spread of the *cfr* gene among pathogens (LaMarre et al. 2011) and underscores the modification of 23S rRNA as a highly effective and transferable form of linezolid resistance (Long and Vester 2012). *Cfr* confers cross-resistance to chloramphenicol/florfenicol, clindamycin, pleuromutilins, and streptogramin B as

these drugs bind to overlapping positions at the ribosomal peptidyl transferase center (Long et al. 2010; Toh et al. 2007). This resistance mechanism draws attention to linezolid's potential cross-resistance with related compounds despite proclamation on its behalf of a new mode of action. Additionally, *cfr* can be linked to *ermB*, a gene responsible for dimethylation of A2058 in 23S rRNA. Coexpression of these two resistance determinants confers resistance to all the clinically relevant antibiotics that target the large ribosomal subunit (Toh et al. 2007). The association with transposon and plasmid genetic elements indicates mobile nature of *cfr* (Toh et al. 2007; Jones et al. 2009b; Diaz et al. 2012) and supports the spread of *cfr*-mediated linezolid resistance across strain and species borders (Feßler et al. 2013; Baos et al. 2013). The frequent association between the cfr gene, mutation G2576T in domain V and mutations in the ribosomal protein L3 cause extremely high level resistance, as observed in *S. epidermidis* (Baos et al. 2013, LaMarre et al. 2013). Thus, it is anticipated that the *cfr* gen together with additional resistance genes (e.g. aminoglycoside resistance genes *aacA-aph*, *aad*, the tetracycline resistance gene *tetL*, the trimethoprim resistance gene *dfrK*, and the macrolide–lincosamide–streptogramin B resistance gene *ermA/B/C*) will be transmitted to other animal and human pathogens, will be co-selected by other antibacterial classes and that multidrug-resistant strains will disseminate and increase in incidence in the near future (Morales et al. 2010; Shen et al. 2013). The report of MRSA with intermediate resistance to glycopeptides, resistance to linezolid, as well as multiple resistances to other second-line antibiotics provides a first glimpse of the challenges yet to be faced (Sorlozano et al. 2010). This is all the more reason to carefully evaluate the linezolid exposure–resistance relationship!

In vitro, the emergence of linezolid-resistant mutants is influenced by the mutational capabilities of the species and the strain. The more frequent occurrence of linezolid failures with enterococci, compared to staphylococci, may also be related to a higher prevalence of the mutator phenotype. In a recent study, the prevalence of the mutator phenotype in *E. faecalis* reached 1.7 %, i.e., about 20 times more than in *S. aureus* (Ba et al. 2010).

Pharmacokinetics/Pharmacodynamics

The selection of an appropriate dose and dosing regimen is a fundamental step for optimizing clinical outcome while reducing selection pressure and minimizing toxicity. Linking drug exposure derived from a dosing scheme (pharmacokinetics) and the exposure–response relationship (pharmacodynamics) in a quantitative way will help to identify the best dosing regimen and thus, clinical outcome (Ambrose et al. 2007). Many in vitro, animal, and clinical studies have described three main MIC-based PK/PD indices that are based on free plasma or serum concentrations (Schmidt et al. 2009): the cumulative percentage of the dosing interval that the free drug concentration exceeds the MIC under steady-state conditions ($t>$MIC); the area under the free concentration–time curve at steady state divided by the MIC (AUC/MIC); and the free peak level divided by the MIC (C_{max}/MIC) (Mouton et al. 2005).

PK/PD in In Vitro and In Vivo Systems

Linezolid has been studied in in vitro systems as well as animal studies to define the PK/PD index that best correlates with outcome.

Andes et al. performed a dose fractionation study using the neutropenic murine thigh infection model and found that the $AUC_{0–24}$/MIC ratio required to produce a static effect against *S. aureus* was 80 for linezolid (Andes et al. 2002). Similarly, Sandberg et al. determined a value of $AUC_{0–24}$/MIC ratio of 100 together with a *ft*>MIC value close to 100 % for maximal effect (Sandberg et al. 2010).

The PK/PD indices of two dosage regimen of linezolid (25 or 50 mg/kg of body weight twice a day) were compared to those of ceftriaxone in an immunocompetent rat model of pneumococcal pneumonia (Gentry-Nielsen et al. 2002). The cumulative mortality rates were 100 % for the control group, 58.3 % for the low-dose linezolid group, 8.3 % for the high-dose linezolid group, and 0 % for the ceftriaxone group. There also were significantly fewer organisms in the ELF of rats treated with ceftriaxone than in the ELF of rats treated with either dose of linezolid. The PK/PD indices predictive of a favorable outcome that is comparable to ceftriaxone were *ft*>MIC>40 % and *f*$AUC_{0–24}$/MIC>150 (Gentry-Nielsen et al. 2002). It should be noted that the values for PK/PD indices might differ in various bacterial species and may be lower for pneumococci than for staphylococci.

In a gerbil model of acute otitis media induced by *S. pneumoniae*, a *t*>MIC of ≥42%, a C_{max}/MIC of ≥3.1, and a $AUC_{0–24}$/MIC of ≥30 was necessary to eradicate *S. pneumonia* (Humphrey et al. 2003).

PK/PD in Humans

Most PK/PD studies in humans have used the approved standard oral or iv dosage regimen of linezolid 600 mg every 12 h and have been focused on ICU patients. In a clinical study of critically ill patients with bacteremia who were enrolled in the compassionate use program, both *t*>MIC and AUC/MIC were highly correlated with outcome (Rayner et al. 2003). According to his data, higher success rates for linezolid may occur at $AUC_{0–24}$/MIC values of 80–120 for bacteremia, lower respiratory infections (LRTI) and SSSI. Chance of success (probability of eradication and clinical cure) in bacteremia, LRTI and SSSI also appear to be higher when concentrations remain above the MIC for the entire dosing interval. Based on this study, both an AUC/MIC of >100 and a *t*>MIC of >85 % have been described as optimal PK/PD targets for clinical efficacy (Rayner et al. 2003).

According to EUCAST, Monte Carlo simulations and target attainment rates for linezolid 600 mg q12h indicate an acceptable rate of target attainment with an MIC of 1.0 (−2.0 μg/mL) (Table 16.3) (EUCAST Steering Committee 2006). A serum PK study in critically ill patients (600 mg q12h) determined a *t*>MIC (MIC 4 μg/mL) of about 11 h (90 % of the dosing interval) and AUC/MIC of 92 (95 % CI 57–128)

Table 16.3 Monte Carlo simulations of target attainment rate for linezolid 600 mg twice daily (EUCAST Steering Committee 2006)

MIC (mg/L)	% Target attainment at AUC/MIC target of		
	50	75	100
≤0.5	100	100	100
1.0	100	100	100
2.0	100	87	83
4.0	75	49	42
8.0	0	0	0

(Whitehouse et al. 2005). Monte Carlo simulations in this study indicated a target attainment rate for an AUC/MIC value of 100 for only 76 % of patients for *S. aureus*, 95.8 % for CoNS, and 75.4 % for *Enterococcus* spp. (Whitehouse et al. 2005).

In critically ill patients, the variability in drug pharmacokinetics may lower the $t>\text{MIC}$ and the AUC/MIC ratio, thus impairing both antibacterial activity and prevention of mutants or induce drug toxicity (Di Paolo et al. 2010). Inadequate linezolid exposure after standard dosage in septic ICU patients was confirmed by C. Adembri et al., who found suboptimal PK/PD values in 40 % of their patients with sepsis and 60 % of their patients with septic shock. The relevant PK/PD values were AUC/MIC (2 μg/mL) ≥ 80 and $ft>\text{MIC}$ (2 μg/mL) ≥ 85 %, respectively (Adembri et al. 2008). Unfortunately, the study was too small to assess the correlation with clinical outcome. To avoid wide fluctuations in serum levels and low trough concentrations, a theoretical advantage of continuous infusion over intermittent infusion in the treatment of infection in critically ill septic patients has been discussed (Adembri et al. 2008). However, there is not enough information about the clinical efficacy of such a dosage regimen that, on the other hand, might favor resistant subpopulations if the concentrations are close to the MIC (Boak et al. 2007).

Hospitalized patients with multiple recent antibiotic exposures are at higher risk for pathogens with elevated MICs. Although these strains may remain designated as susceptible by accepted MIC break points, such elevated MIC values may compound a potentially impaired PK situation and shift the desired PK/PD ratio to higher necessary drug–exposure values than are achievable due to drug–toxicity limits. For drugs, such as linezolid, with relatively high MICs in wild-type populations that cluster around the breakpoint, fluctuations in PK create variable drug exposure conditions, potentially resulting in failure to reach the PK/PD target (Theuretzbacher 2012).

Various PK/PD aspects at the site of infection have been investigated in healthy volunteers and patients. In healthy volunteers, the AUC_{0-24}/MIC for free linezolid in the IF of subcutaneous adipose and skeletal muscle tissue was found to range between 50 and 100 for pathogens with MICs between 2 and 4 mg/L (Dehghanyar et al. 2005). However, as has been shown in other studies, large interindividual differences in the pharmacokinetics of linezolid exist (Dehghanyar et al. 2005). Case in point, a similar study in patients confirmed the uncertainty of effective concentrations being achieved in the IF for a sufficient time period in individual patients (Buerger et al. 2006). In this study, one-third of patients showed $ft>\text{MIC}$ values in plasma of less than 40 % and most patients had a $f\text{AUC/MIC} \leq 50$ with a

concordant increased risk of inadequate drug exposure (Buerger et al. 2006). Concentrations in ELF and alveolar macrophages have been determined. For a MIC of 4 μg/mL, the $AUC_{0–12}$/MIC ratio was 35 in plasma and 120 in ELF, and the $t>MIC$ for the 12-h dosing interval was 100 % in plasma and ELF (Conte et al. 2002). Unfortunately, no correlations of PK/PD with clinical outcome in patients with pneumonia exist.

In the absence of new drug candidates in late-stage clinical development for tuberculosis existing drugs are tested for their usefulness in multidrug-resistant tuberculosis. Linezolid is one of these drugs and has been evaluated in several studies. Despite adequate linezolid exposure in TB patients after 600 mg q12h (McGee et al. 2009), the clinical effectiveness of linezolid has not been established. Linezolid had modest early bactericidal activity and little extended bactericidal activity (Dietze et al. 2008). No correlation was found between activity and *f*AUC/MIC or $t>MIC$ (Dietze et al. 2008).

PK/PD and Resistance

PK/PD parameters that predict efficacy have been well described for all groups of antimicrobials, whereas knowledge concerning PK/PD parameters that might additionally correlate with selection of resistance is still in its infancy. In the body, gradients of fluctuating antibiotic concentrations are formed at several sites and diverse antibiotic selective pressures are created that affect not only the infective pathogens but also the commensal flora. The relationship between drug dosage and resistance development, determined on the basis of PK/PD properties that prevent emergence of preexisting or newly formed mutants, is increasingly under investigation (Olofsson and Cars 2007).

Dynamic in vitro models that simulate in vivo concentration profiles are helpful for determining the PK/PD index that may be most predictive for suppressing resistance. Several studies have shown the influence of the PK/PD index AUC/MIC on the emergence of linezolid resistance. Using high inoculum, longer duration of the experiment, and using a variety of different strains (including hypermutators) mimics certain basic clinical situations such as infections with high bacterial load (e.g., pneumonia, endocarditis), infections with long duration of therapy (endocarditis, osteomyelitis), or infections with a higher likelihood of hypermutator strains (e.g., enterococci). Simulating dosing regimens that mimic constant concentrations in the vicinity of the MIC of the bacteria (e.g., continuous infusion) produces increases in MICs and substantial changes in the population analysis profiles (PAPs) (Boak et al. 2007). The PAP method detects changes in susceptibility within subpopulations through the use of multiple sub- and supra-MICs of the chosen antibiotic.

The longer duration of the experiments mimics more closely the clinical situation. A model that simulated a twice-daily linezolid regimen for 5 consecutive days with a broad range of simulated $AUC_{0–24}$/MIC ratios found a bacterial regrowth that followed a pronounced reduction of the starting inoculum at each simulated $AUC_{0–24}$/MIC ratio. The times to regrowth tended to be shorter at lower $AUC_{0–24}$/MIC values

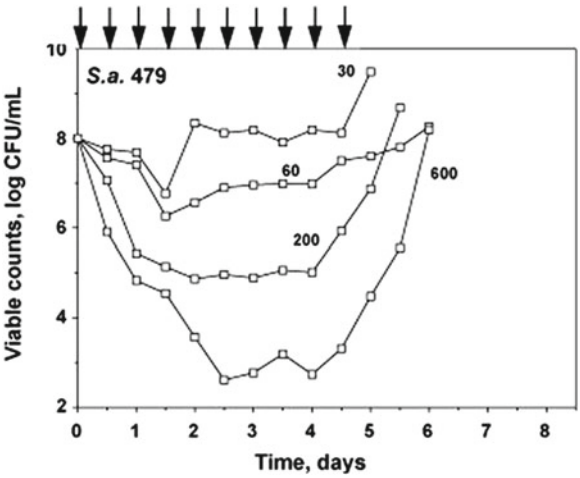

Fig. 16.9 Kinetics of killing and re-growth of *Staphylococcus aureus* 479 (MRSA) exposed to linezolid (modified Strukova et al. 2009, reproduced with permission). The simulated 24-h area under the concentration–time curve to minimum inhibitory concentration ratio (AUC24/MIC) in hours is indicated by the number at each curve. The *arrows* reflect antibiotic doses. *CFU* colony-forming units

(Fig. 16.9) (Strukova et al. 2009). With a linezolid dosage regimen of 600 mg twice daily mean $AUC_{0–24}$/MIC values of 100–130 would be achieved assuming a *S. aureus* MIC of 2 μg/mL. Lacking the factor immune system, in this in vitro model all simulated regimes failed to prevent regrowth. Other PK/PD indices have been implicated in predicting resistance development. For *Bacillus anthracis*, resistance prevention was linked to the C_{max}/MIC ratio (Louie et al. 2008). Zinner et al. suggested an $AUC_{0–24}$/MIC ratio >200 to protect against the selection of linezolid resistance in enterococci (Zinner et al. 2008).

As mentioned above, strain-specific characteristics play an important role in the risk of developing resistance. Bacterial mutator phenotypes are readily selected in in vitro systems. *E. faecalis* is known for its higher prevalence of the mutator phenotype (Ba et al. 2010). The emergence of highly resistant mutants could not be prevented in an in vitro system simulating either the standard 600-mg or a 800-mg linezolid dosage against a clinical *E. faecalis* mutator phenotype strain (Ba et al. 2010).

Results of in vitro studies have been confirmed in the clinical situation. Patients in the compassionate-use study who developed decreased susceptibility to linezolid (fourfold or greater increases in the MIC) during treatment also exhibited AUC/MIC and $\%t > \text{MIC}$ values <100 (Rayner et al. 2003). As shown in Cystic Fibrosis patients, extended periods of linezolid exposure increase the risk of emergence in *S. aureus* (Endimiani et al. 2011).

For quinolones, a concept of the mutant selection window (MSW) (Fig. 16.10) has been proposed that describes a concentration range in which selective amplification of single-step, drug-resistant mutants occurs (see review Drlica and Zhao 2007). The MSW is defined by the antibacterial concentration curve and the concentration range between MIC and mutant prevention concentration (MPC—concentration that prevents the amplification of resistant mutants). This concept has been also applied to linezolid, with the assumption that concentrations exceeding the MPC and below the MIC should rarely select for resistant subpopulations. The clinical

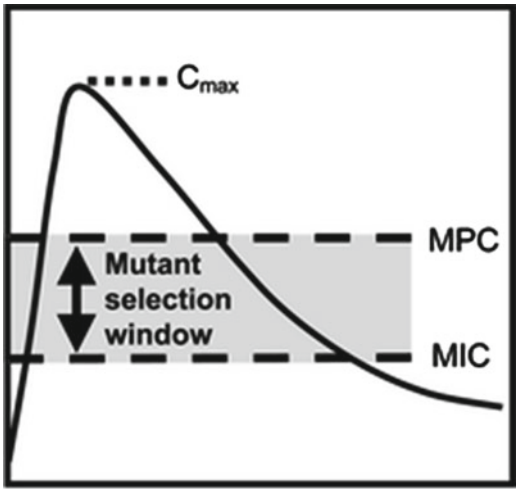

Fig. 16.10 Schematic representation of the selection window (Drlica and Zhao 2007), reproduced with permission

relevance and the cutoff values for the resistance related PK/PD indices, such as C_{max}/MPC, AUC/MPC or %tMSW (% of each dosage interval that concentrations fall within the MSW), and %t>MPC (% of the dosage interval that concentrations exceed the MPC), are ill defined.

In *E. faecium*, selection of linezolid-resistant mutants was observed when the %tMSW was either 100 % or 70 %, but not when %tMSW was 0 %. On the other hand, a linezolid AUC/MIC of 230 protected against linezolid resistance (Zinner et al. 2008). In this study, the simulated concentration range was not large enough to find the cutoff value for the parameter %tMSW. The same study found that coadministration of doxycycline protected against the development of linezolid resistance (Zinner et al. 2008). In an in vitro model simulating a 600 mg q12h linezolid regime against *E. faecium* and *E. faecalis* (MIC 2 μg/mL, MPC 4–8 μg/mL), AUC/MPC values of about 8 (%tMSW = 80 %) and 15 (%tMSW = 40 %), respectively, had been achieved which allowed linezolid-resistant subpopulations to be selected (Allen and Bierman 2009). These data confirm that linezolid resistance is readily selected upon exposure to linezolid concentrations within the MSW. The current dosage regime of linezolid as monotherapy will not protect against resistance selection in enterococci and emergence and dissemination of linezolid resistance is anticipated. Additionally, the concept of selection of resistant mutants within the MWS has been recently challenged as the selection may also occur at extremely low antibiotic concentrations (Gullberg et al. 2012).

Further studies evaluating a range of linezolid exposures are necessary to adequately describe the pharmacodynamics of linezolid resistance.

The risk of resistance development is lower in *S. aureus* compared to enterococci. G.P. Allen et al. used concentrations according to the approved dosage regimen and calculated several resistance PK/PD indices in five community and hospital acquired MRSA strains. The MPC/MIC ratio was 4–8, the AUC/MPC 6–12, %tMSW 70–100 %, and %t>MPC 0–25 % (Allen and Deshpande 2010).

The values of these indices that best predict the suppression of resistant mutants of *S. aureus* is not yet known.

Off-label and low-dose usage of linezolid in multidrug regimens against multidrug-resistant tuberculosis (Koh et al. 2012) might spur resistance development in the commensal flora with unknown consequences for the future resistance situation of oxazolidinones in general (Bolhuis et al. 2012). Due to long-term treatment requirements and associated potential of increased toxicity, there is a trend to reduced dosage regimens. Without TDM and, thus observing actual drug exposure in individual patients while also considering the MIC of the bacterial isolate, reducing linezolid dosage carries a high risk of development of resistance.

Due to the huge variety in drug exposure in patients, an optimal resistance-preventing dosage regimen may be limited by the PK and toxicity of the drug. However, this may be circumvented using combination therapy (Olofsson and Cars 2007). Preliminary in vitro studies show that the emergence of resistant mutants can be delayed when combined with other antibiotics of several groups even at sub-MIC concentrations (Miller et al. 2008). A number of questions remain regarding how to identify best regimen for suppression of resistant mutants while minimizing toxicity. Combination regimens have yet to be evaluated in carefully designed clinical studies.

PK/PD and Toxicity

In phase III clinical trials, only minor adverse effects were seen in linezolid-treated patients (Butterfield et al. 2012). However, numerous more serious adverse effects were reported after commercial release, including thrombocytopenia, lactic acidosis, peripheral and optic neuropathy, and serotonin syndrome (Narita et al. 2007; Jang et al. 2009; Woytowish et al. 2013). A general feature of the oxazolidinone class of antibiotics is the inhibition of mammalian mitochondrial protein synthesis effected by binding to mitochondrial ribosomes in all tissues (Barnhill et al. 2012). As mentioned earlier, this effect is not surprising considering the similarities between bacterial ribosomes and mammalian mitochondrial ribosomes (Denslow and O'Brien 1978). Typical therapeutic doses of linezolid yield blood and tissue levels that are at or in some cases above the IC_{50} values for inhibiting mitochondrial protein synthesis (Fig. 16.11). Indeed, a dose- and time-dependent decrease of mitochondrial respiratory chain enzyme activity at therapeutic concentrations has been observed in patients (De Vriese et al. 2006). Consistent with these results, dose-dependent and reversible bone marrow suppression, typically thrombocytopenia, has been noted as a side effect of treatment with linezolid (also seen with chloramphenicol and other ribosome binding antibiotics) (McKee et al. 2006; Matsumoto et al. 2010). The effects of linezolid exposure on thrombocytopenia, as well as the relationship between renal function, liver cirrhosis, and drug exposure, were recently investigated (Sasaki et al. 2011; Kazuaki et al. 2010; Tsuji et al. 2011). Compared to nonthrombocytopenic patients with normal renal function, the linezolid exposure was about twice as

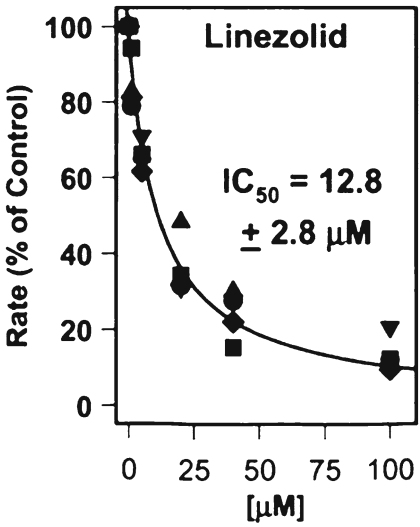

Fig. 16.11 Dose–response effect of linezolid on mitochondrial protein synthesis. Isolated heart mitochondria were incubated with various concentrations of linezolid. Each symbol represents a separate experiment (modified McKee et al. 2006, reproduced with permission)

high in patients with thrombocytopenia and renal dysfunction (trough concentrations ≥14.4 µg/mL and AUC_{0-24} ≥513.1 mg h/L) (Kazuaki et al. 2010). Similar results were obtained in a recent study that determined a threshold for C_{min} above 10 µg/mL as a risk factor for hematological toxicity (Cattaneo et al. 2013). Creatinine clearance <50–60 mL/min has been identified as a major risk factor for thrombocytopenia (Nukui et al. 2013) with a significantly shorter time to the onset of thrombocytopenia compared to patients with creatinine clearance ≥50 mL/min (Takahashi et al. 2011). In renal insufficiency, the accumulation of the two metabolites with no antimicrobial activity but unknown toxicity has been connected with mitochondrial toxicity. Clinical studies have shown that increased AUC as well as duration of therapy correlated with decreased platelet count and hemoglobin levels (Figs. 16.12 and 16.13) (Forrest et al. 2000; Tsuji et al. 2011). Tsuji et al. suggest using hemoglobin levels as an index of the development of linezolid-associated thrombocytopenia (Tsuji et al. 2011) while keeping in mind the substantial variability in individual sensitivity to linezolid as shown by reversible immediate hematological toxicity after a single dose of linezolid (Cai et al. 2012). Similarly, Hiraki et al. demonstrated that trough concentrations correlated with platelet counts. Specifically, a significant decrease in platelet count was observed in patients with trough linezolid concentrations higher than 22.1 µg/mL (Hiraki et al. 2012).

Linezolid-induced mitochondrial toxicity also causes clinical signs of peripheral neuropathy, hyperlactatemia, and metabolic acidosis by reducing mitochondrial respiratory chain enzyme activity (Casanova-Molla et al. 2012; Garrabou et al. 2007). Though generally rare, these adverse events are dose and time dependent, thus, typically associated with prolonged courses. Off-label long-term treatment of multidrug-resistant tuberculosis is known for its significant adverse events with almost 40 % of patients discontinuing linezolid due to severe side effects (Cox and

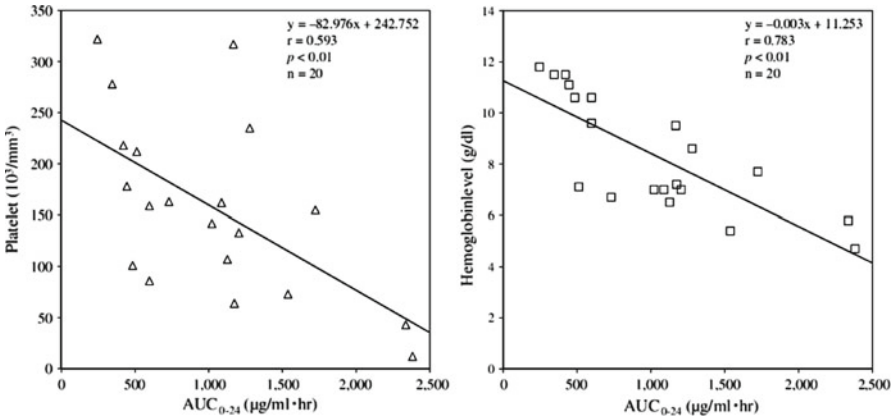

Fig. 16.12 Correlations of linezolid AUC_{0-24} with platelet count and hemoglobin level (Tsuji et al. 2011), reproduced with permission

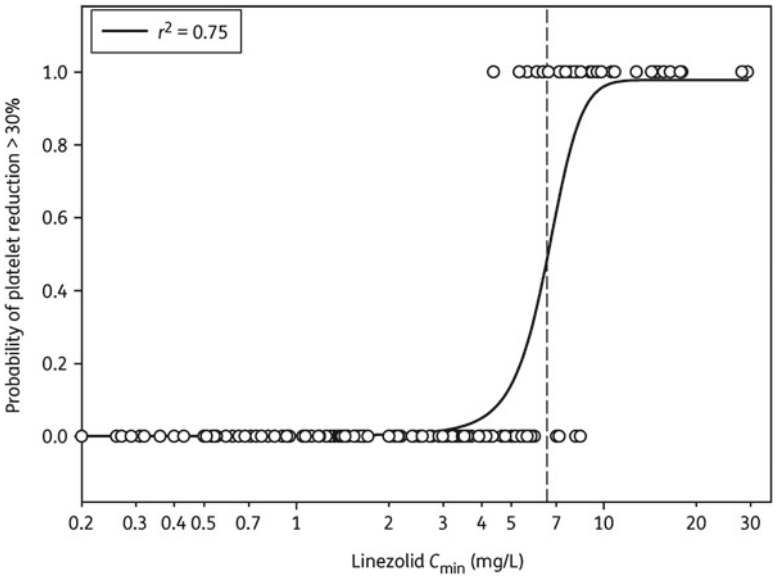

Fig. 16.13 Linezolid C_{min} and logistic regression model for thrombocytopenia (Pea et al. 2012), reproduced with permission. The symbols refer to the C_{min} observed over time in each patient with (*top*) or without (*bottom*) thrombocytopenia. The *continuous line* represents the result of the logistic regression model. The *vertical broken line* identifies the C_{min} value predicting 50 % probability of thrombocytopenia

Ford 2012) and thus, confirming the cumulative concentration effect of the drug (Jang et al. 2009; Beekmann et al. 2008). More studies are needed to elucidate the mechanism of toxicity in relation to drug exposure.

Therapeutic Drug Monitoring

Despite numerous studies showing insufficient drug exposure in severely ill patient groups, TDM and individualized dosing in the daily clinical routine (Di Paolo et al. 2010; Alffenaar et al. 2010) is not well defined. As described in the pharmacokinetics chapter, substantial interpatient and sometimes intrapatient variability in critically ill patients makes it impossible to accurately predict the pharmacokinetic disposition of linezolid in individual patients following the standard dosing regimen of 600 mg twice daily. Instead, individual pathophysiological conditions determine the exposure of linezolid. Therefore, TDM in vulnerable patients might be especially valuable in increasing efficacy when faced with MIC values of >0.5 µl/mL and lowering the risk of exposure-dependent toxicity.

Dong et al. highlighted the importance of individual monitoring of linezolid exposure and MICs to target the dosages to individual patient's specific properties, while showing that the standard fixed dosage of 600 mg twice daily may result in adequate exposure to linezolid in only 60–70 % of critically ill patients (Dong et al. 2011). Another study confirms these results while emphasizing that the risk of potential linezolid overexposure and associated toxicity (Pea et al. 2010) could be minimized with TDM.

With linezolid, the clear linear relationship between C_{min} and estimated $AUC_{0–24}$ suggests that C_{min} may represent a useful predictor of total body exposure in daily clinical practice (Pea et al. 2012). Considering a PK/PD target of C_{min} higher than MIC_{90} and an AUC/MIC_{90} ratio of >100, an approximate toxicity threshold of C_{min} 10–15 µg/mL, and an achievable AUC_{0-24} (in the presence of C_{min} of >2 mg/L) of >160 mg h/L, a TDM threshold of C_{min} between 2 and 7 mg/L and/or of AUC_{0-24} between 160 and 300 mg/L h may improve safety outcomes while retaining appropriate efficacy (Pea et al. 2012). This is especially the case in patients receiving prolonged linezolid treatment and patients with impaired kidney function (Pea et al. 2012; Nukui et al. 2013).

TDM-guided dosage adjustments are especially necessary in critically ill patients with unpredictable drug exposure, in patients with peculiar pathophysiological conditions, in patients with infections who require prolonged treatment, and in patients cotreated with P-gp modulators (i.e., omeprazole, amiodarone, clarithromycin, and rifampicin) (Pea et al. 2005, 2010, 2012; Sousa et al. 2011; Yagi et al. 2013).

Dosing Implications

During the drug development process for linezolid, dose finding and dose confirming studies focused primarily on regulatory and business requirements. However, following approval a wealth of additional studies have included additional perspectives and a wide range of patient populations. As a result, aspects other than noninferiority clinical endpoints compared to older antibiotics in homogenous patient

populations have garnered attention. Specifically, new knowledge has supported moving from a one-size-fits-all dosing approach to individualized dosing based on TDM thus providing a higher probability of clinical success and improved toxicity controls. Additionally, consideration of interactions with P-glycoprotein-sensitive drugs, of adaption of dosages in case of insufficient renal function or severe liver cirrhosis has been introduced into clinical daily life.

While new dosing concepts are currently being investigated, more studies and data are needed. One new concept is the use of continuous infusion to reduce the pharmacokinetic variability in selected severely ill patients (Theuretzbacher 2012). Despite a few pharmacokinetic studies (Adembri et al. 2008; Boselli et al. 2012) and in vitro models (Boak et al. 2007), the clinical benefit of continuous infusion still needs to be determined as clinical data are not conclusive. Indeed, there is some evidence that continuous infusion of linezolid may promote the selection of resistant subpopulations as shown in in vitro models (Boak et al. 2007).

Another innovative dosing concept utilizes a "front-loading" regimen with the administration of high doses early in therapy for a short duration (Tsuji et al. 2012b). In this protocol, the in vitro model showed that front-loading may provide additional killing for some, but not all, strains and may delay, but not prevent, the emergence of resistance. The potential for toxicity due to increased cumulative exposure could not be determined in this model and clinical information does not exist.

PK/PD and New Oxazolidinones

The group of oxazolidinones provides an attractive target for modification of the molecule and improvement of important characteristics such as broadening the antibacterial spectrum to cover *Haemophilus influenzae*, reestablishing sensitivity to linezolid-resistant strains and lowering the toxicity potential. Though considerable challenges in discovery of new oxazolidinones remain, several linezolid-analogs have been described that are in preclinical or clinical development (Srivastava et al. 2008). The most advanced of these compounds is tedizolid. A New Drug Application has been filed in October 2013.

Tedizolid (Cubist Pharmaceuticals)

As expected, tedizolid displays pharmacological properties comparable to linezolid. The main improvement over linezolid is tedizolid's higher intrinsic activity as denoted by an approximately four times lower MIC in wild-type strains when compared to linezolid and a longer half time allowing QD dosing (Livermore et al. 2009; Schaadt et al. 2009; Muñoz et al. 2010; Prokocimer et al. 2012; Rodríguez-Avial et al. 2012). It should be noted that the MIC determinations were carried out in protein-free media. Due to a plasma protein binding rate of about 90 %, the MIC values increase fourfold when tested in 80 % complement-inactivated human sera

(Louie et al. 2011; Housman et al. 2012). The suggested lower breakpoint of 0.5 μg/mL compared to linezolid (Keel et al. 2012b) mirrors the effect of the protein binding. Similar to linezolid, the *f*AUC/MIC ratio is the pharmacodynamic index that best correlates with treatment effect. A *f*AUC/MIC ratio of about 70–75 is required to achieve 1 log CFU/g reduction of S. aureus (Louie et al. 2011).

A major improvement target in the oxazolidinone class is the coverage of linezolid-resistant strains. As expected, there is a positive correlation between the number of mutated copies of the 23S rRNA gen and the MICs of both linezolid and tedizolid (Livermore et al. 2009). Due to its improved intrinsic activity compared to linezolid (Schaadt et al. 2009), tedizolid's MIC increase may still stay in the susceptible range in *S. aureus* strains with limited mutated gene copies. While no official breakpoints are available yet, it is anticipated that this benefit may not apply to enterococci with ≥2 mutated gene copies (Livermore et al. 2009).

In the immunocompetent murine thigh model, human simulated exposures of tedizolid and linezolid after 200 mg QD and 600 mg BD, respectively, resulted in similar efficacy against both methicillin-susceptible and resistant *S. aureus* (Keel et al. 2012b). While both agents are recognized as having bacteriostatic activity at 24 h, their activity is enhanced over time (Keel et al. 2012b). Utilizing a neutropenic mouse thigh model of staphylococcal infection, tedizolid demonstrated a markedly lower effect (about 20-fold) in the absence of granulocytes in reducing the bacterial load. This mouse thigh model showed that the antistaphylococcal activity of tedizolid based on human exposures depends on the presence of granulocytes (Drusano et al. 2011 September). The mechanism behind this granulocyte-mediated enhancement of tedizolid remains unclear.

In vitro studies reveal that tedizolid's intracellular penetration into PMNs and epithelial cells is rapid and reaches concentrations ~10- to 15-fold the extracellular concentration but is highly influenced by pH and temperature (Lemaire et al. 2009). The higher accumulation of torezolid is quantitatively offset by a commensurate decrease in its activity in the intracellular milieu (Lemaire et al. 2009). In terms of relative efficacy (equivalent multiples of the MIC), the intracellular activity of tedizolid is similar to linezolid (Lemaire et al. 2009) and, so, does not explain the marked effects in the animal model. The immunocompetent pneumococcal thigh and lung infection model showed improved survival based on equivalent doses (Choi et al. 2012).

In drug development, determining the optimal therapeutic dose for phase III trials is a crucial step. In contrast to former protocols, when the dose finding was based on trial and error, modern drug development utilizes PK/PD modeling, phase I/II safety and PK, as well as efficacy results from phase II dose-ranging trials as key criteria for selecting the appropriate dose. Tedizolid has provided a good example of a rational dose finding approach to optimize efficacy based on population PK/PD modeling and simulation using data from mice and the population PK data from healthy volunteers (Prokocimer et al. 2011). Though optimized for microbiological and clinical efficacy in noncritically ill patients, the appropriate dose regimen for severely ill patients as well as minimizing emergence of resistant mutants is not known and not considered.

PK data from phase I studies in healthy volunteers indicated concentrations above the MIC 0.25 μg/mL with a single dose over 24 h and no accumulation (Bien et al. 2010a). However, a wide interpatient variety in PK data was also seen in these studies. The high protein binding of about 90 % was not considered when relating total concentrations (protein bound + free) to testing in protein-free media. The mean (SD) plasma parameters in healthy volunteers after 7 days of tedizolid iv 200 mg QD administration is 3 μg/mL (0.66) for C_{max}, 12 h (1.3) for $t_{1/2}$, 29 μg h/mL (6.2) for $AUC_{0–24}$, 5.9 L/h (1.4) for CL, and 80 L (Meagher et al. 2003) for V_{ss} (Bien et al. 2010b). Tedizolid half-life values were approximately 2-fold greater compared with linezolid (Flanagan et al. 2013b). Again, these pharmacokinetic values represent total concentrations including the bound and free fraction of the drug. Similar to linezolid, the absolute bioavailability (>90 %) of the oral form allows the interchangeability of oral and iv forms (Bien et al. 2010b). A microdialysis study in healthy volunteers confirmed the free concentrations in the interstitial fluid of muscle and adipose tissue to be comparable to the free concentrations in plasma with a mean total $AUC_{0–24}$ of 57.1 μg h/mL and a mean $fAUC_{0–24}$ of 7.3 μg h/mL (Sahre et al. 2012). The penetration into both the ELF and AM compartments of the lung in healthy volunteers and the $AUC_{0–24}/MIC_{90}$ exposures were similar to linezolid (Housman et al. 2012). In vitro studies confirmed that, like linezolid, tedizolid is a reversible inhibitor of MAO-A and MAO-B. Due to lower free drug concentrations and shorter duration of therapy the potential for physiologically relevant MAO interactions may be lower compared to linezolid (Flanagan et al. 2013a).

The safety profile in healthy volunteers indicated limited dose-dependent effects on platelet counts over the 21-day study (Muñoz et al. 2010). This reduction of platelet counts was not seen in the first week of treatment. Population analysis complemented the in vitro, in vivo, and phase II clinical trial results and resulted in a PK/PD analysis that confirmed the planned phase III dosing regimen of 200 mg QD for 6 days in acute bacterial skin and skin structure infections (ABSSSI) as the minimally effective dose in non-severely ill patients. Two Phase 3 studies in ABSSSI confirmed the non-inferiority to linezolid in patients with cellulitis/erysipelas, major cutaneous abscess, or wound infection due to susceptible organisms (Prokocimer et al. 2013). The exploration of the exposure/resistance relationship as well as dosing in severely ill patients and in patients with impaired immune system should be a focus of future studies. It is anticipated that higher doses will be necessary in clinical practice when considering the high PK variability in inhomogeneous patient populations.

Radezolid (Melinta Therapeutics)

This new linezolid analog, radezolid, binds more tightly to ribosomes than linezolid and has slightly lower MIC values (Skripkin et al. 2008). It is not known yet to what extent the high protein binding (about 97 %) will offset this improved activity as, according to available publications, the in vitro activity has been measured only in protein-free media. Additionally, it is not known whether the higher intracellular

penetration measured in vitro (Lemaire et al. 2010a) and in vivo (Burak et al. 2009; Lemaire et al. 2010b) might translate to better in vivo activity. The MIC values of radezolid in staphylococci are usually similar to tedizolid within one or two dilution steps. According to in vitro tests with genetically defined strains, the activity of radezolid seems to be marginally better than tedizolid in 23S rRNA mutants with the opposite observation in *cfr* containing strains (Farrell et al. 2011b). Again, all of these MIC tests were carried out in protein-free media and the reduced activity in blood might shift MICs of 1–4 μg/mL in linezolid-resistant strains to values that are beyond concentrations achievable in humans. Radezolid has been tested in community-acquired pneumonia and in uncomplicated skin and soft tissue infections in two phase II studies. No advancement to phase III clinical trials has been reported.

MRX-I (MicuRx Pharmaceuticals)

MRX-1 is tested in Phase II clinical studies. It remains to be seen if the slightly lower MIC values compared to linezolid (protein binding not published) and the improved safety profile translates into a meaningful benefit for patients.

LCB01-0371 (LegoChem Biosciences)

This oxazolidinone with cyclic amidrazone has advanced to Phase I clinical studies. In preclinical in vitro and in vivo studies LCB01-0371 was as active as linezolid.

Outlook

Linezolid has been clinically available for more than 10 years and we can reference a substantial volume of PK/PD data that aim at optimizing its dosing. Nevertheless, three primary areas require additional investigation before we can tap the full potential of the oxazolidinone class of antibacterial drugs.

- Wide interpatient variability remains a serious obstacle to the treatment of critically ill patients with linezolid. Treatment optimization and individualized dosing based on TDM should be considered a requirement and implemented for more effective and tolerable use of linezolid, particularly in these vulnerable patient populations.
- Considering the ever-increasing gap between the growth of resistance and the lack of new antibiotics without cross-resistance (Theuretzbacher 2009; Theuretzbacher 2013), all strategies targeted at preventing an increase of resistance against the valuable group of oxazolidinones must be intensely pursued. An important step in this regard would be the diligent definition of exposure/resistance relationships aimed at optimizing dosing to minimize likelihood of resistance emergence.

- Especially, important for patients who need long-term treatment, a better understanding of the mitochondrial toxicity of oxazolidinones correlated with cumulative exposure will likely improve the safe usage of the drug.

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Chapter 17
Tigecycline

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Abstract Tigecycline is a glycylcycline antibiotic with broad-spectrum activity against Gram-positive, Gram-negative, atypical, anaerobic and multidrug resistant organisms. Tigecycline displays linear pharmacokinetics with widespread tissue distribution into both infected and non-infected tissue and non-linear protein binding. Tigecycline has a half-life between 40-60 hours and is mostly eliminated unchanged as parent drug in the feces, with minor elimination via the urine. Free-drug AUC:MIC ratio is the PK-PD index most closely associated with the efficacy of tigecycline. Dosing of tigecycline does not need to be adjusted for those patients with renal insufficiency. However, those patients with severely decompensated cirrhosis of the liver may require a dosing adjustment. The most commonly reported adverse events associated with the use of tigecycline include nausea and vomiting and are linked to high 24 hour AUC values.

Keywords Tigecycline • Broad-spectrum • Protein synthesis inhibitor

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Introduction

Tigecycline (Pfizer®, Pfizer Inc., New York, NY) is a member of the glycylcyclines group of antibiotics and is structurally related to the tetracyclines. It is a broad-spectrum agent, which displays activity against a range of Gram-positive, Gram-negative, atypical, anaerobic, and multidrug-resistant organisms; however, tigecycline does not possess clinically significant activity against *Pseudomonas aeruginosa* or *Proteus* species (Zhanel et al. 2004). Tigecycline is an analogue of minocycline and similar to minocycline, it binds to the 30S ribosomal subunit of bacteria, blocking the entry of aminoacyl transfer RNA molecules into the acceptor site of the ribosome. This prevents the incorporation of amino acid residues into the peptide chain, which inhibits protein synthesis (Chopra and Roberts 2001). This ribosomal binding is reversible, lending to bacteriostatic activity (Chopra and Roberts 2001).

Bacterial resistance to the tetracyclines occurs through the acquisition of tetracycline resistance genes (*tet*-genes), which are encoded on transferable genetic elements that allow for the transfer between species (Zhanel et al. 2004). These *tet* genes encode for either efflux pumps, which pump the tetracyclines out of the bacterial cell, or ribosomal protection. Tigecycline is active against isolates which have *tet*-protected ribosomes through the proposed ability to bind more tightly to the ribosome so that the product of the *tet*-gene cannot interfere with the ribosome (Zhanel et al. 2004). Additionally, tigecycline is active against organisms that display efflux-mediated resistance by either being unable to induce the efflux protein or because the protein cannot export the agent (Zhanel et al. 2004).

Pharmacokinetics

Tigecycline is available only as a parenteral formulation due to limited oral bioavailability. The pharmacokinetics (PK) of tigecycline have been studied in healthy volunteers as well as in special populations, including patients with cirrhosis.

Tigecycline has been shown to display linear pharmacokinetics after ascending single doses and at steady state after multiple doses (Muralidharan et al. 2005). In healthy volunteers administered single doses of tigecycline ranging between 12.5 and 300 mg given as 1-h infusions, the maximum serum concentration (C_{max}) ranged between 0.11 and 2.82 μg/mL (Muralidharan et al. 2005). Subjects in this single-ascending dose study were administered doses under fasting and fed conditions and tigecycline pharmacokinetics did not vary between states; however, administration under a fed state seem to result in better tolerability of higher doses (Muralidharan et al. 2005).

Multiple studies have demonstrated that tigecycline has rapid and extensive distribution into body tissues. Studies in healthy volunteers show that at steady state, the volume of distribution varies between 7 and 10 L/kg, which indicates widespread tissue distribution (Muralidharan et al. 2005). An inflammatory blister fluid

study conducted in healthy volunteers was used to evaluate the distribution of tigecycline into skin tissue. This study showed that tigecycline demonstrated a 74 % tissue penetration ratio. These results confirmed and translated previous radiolabeled work completed in rats which initially determined that tigecycline distributes extensively into tissues (Sun et al. 2005; Tombs 1999). In addition, this blister study also showed that tissue distribution is rapid with the peak concentration seen within 3 h of drug administration (Sun et al. 2005). A single-dose intravenous study in subjects undergoing medical or surgical procedures displayed the extent of this tissue distribution by demonstrating that tigecycline achieved high tissue fluid concentrations in bile, gallbladder, colon, and lung tissues when compared with simultaneously obtained serum concentrations (Rodvold et al. 2006). Data are limited on the distribution of tigecycline into infected tissues; however, recent data obtained from patients with diabetic lower limb infections reveal that the distribution of tigecycline into infected and noninfected tissue is similar with tissue penetration ratios of 100 % and 99 %, respectively (Bulik et al. 2010). Tigecycline has been shown to display nonlinear protein binding in human plasma in vitro, but opposed to other agents who also display nonlinear protein binding, the free fraction of tigecycline has been shown to decrease as concentrations increase (Muralidharan et al. 2005). While this unique protein binding has been confirmed in vivo in a murine thigh infection model, not until recently has this atypical protein binding been confirmed over changing concentrations and time in the same patient (Crandon et al. 2009a; Bulik et al. 2010). The study by Bulik et al. also demonstrated that this tissue penetration occurs very rapidly, within 4 h in both infected and uninfected tissues (Bulik et al. 2010). Many theories have been proposed to explain the mechanism for this atypical protein binding. The most common theory is the ability of tigecycline to form chelate complexes with multivalent metal ions that bind to plasma proteins, a property similar to tetracycline (Muralidharan et al. 2005; Chopra and Roberts 2001). These chelate complexes affect diffusion rates across semipermeable membranes and also affect binding to cellular proteins (Muralidharan et al. 2005).

The metabolism and elimination of tigecycline was evaluated in a study involving healthy male volunteers administered a single 100 mg dose followed by six 50 mg doses, with the final dose administered as a radiolabeled dose (Hoffman et al. 2007). The results of this study identified that tigecycline circulates in the plasma mainly as parent drug, and mostly parent drug was collected from urine and feces. Tigecycline's major metabolic pathways were identified as glucuronidation and amide hydrolysis followed by *N*-acetylation (Hoffman et al. 2007). This study also identified the primary route of elimination of unchanged tigecycline to be via the feces. The secondary elimination pathways were identified as renal excretion and metabolism by glucuronidation and *N*-acetylation (Hoffman et al. 2007). The extent of renal elimination was quantified in healthy volunteers in a single ascending dose study. This study showed that less than 13 % of tigecycline was excreted in the urine as unchanged drug (Muralidharan et al. 2005). Additionally, the mean clearance of tigecycline was quantified as 0.2–0.3 L/h/kg and was not found to be dose dependent. Finally, the elimination half-life of tigecycline was found to range between 40 and 60 h (Muralidharan et al. 2005).

Special Populations

Renal Insufficiency

A single-dose study in patients with severe renal impairment or end-stage renal disease, both on and not on hemodialysis, was conducted to examine whether tigecycline pharmacokinetics were altered in these populations (Troy et al. 2003). All subjects were administered a single dose of 100 mg infused over 1 h; however, in patients undergoing hemodialysis, the dose was administered 2 h before or 4 h after the hemodialysis session in order to evaluate if the drug was dialyzable. When the results of this study were compared to the age-matched healthy controls, despite the mean tigecycline peak concentration being 60 % higher in patients with ESRD, the AUC was only 20 % higher in these subjects. In subjects with severe renal impairment, the tigecycline peak concentrations were similar to those of the healthy controls; however, the mean AUC values were 40 % higher. Despite these numerical differences, no statistical differences were found and a higher incidence of adverse events was also not found. In subjects undergoing hemodialysis, administration of tigecycline before or after the session did not affect the pharmacokinetics of the drug, indicating that tigecycline is not cleared through hemodialysis. These data suggest that tigecycline may be administered to hemodialysis subjects without regard to their hemodialysis schedule and furthermore, that tigecycline does not require dose adjustment for renal function (Troy et al. 2003; Zhanel et al. 2004).

Hepatic Insufficiency

The pharmacokinetics of tigecycline in subjects with hepatic impairment was evaluated in a single-dose, case-controlled study (Korth-Bradley et al. 2011). In this study, 25 healthy subjects were matched in age, sex, dry weight, and smoking status with 25 subjects who had biopsy-proven cirrhosis. All patients were administered a single intravenous dose of tigecycline 100 mg over 60 min and pharmacokinetic samples were taken up to 120 h after the infusion. The results showed that starting 1.5 h after the drug administration, serum tigecycline concentrations were higher in subjects with severely decompensated cirrhosis than in other groups of subjects. Overall, subjects that had moderately decompensated cirrhosis (Child-Pugh B) had much different PK values than the matched controls such that tigecycline clearance was 25 % lower, AUC was 50 % higher, half-life was 25 % longer, and the amount of unchanged tigecycline that was excreted in the urine was 53 % higher than the controls. Despite these differences, when simulation and modeling was applied to include these results, a dosage adjustment was not necessary in the group due to the acquisition of a suboptimal AUC with a reduction in the maintenance dose. This finding was not reciprocated in the severely decompensated cirrhosis group. The differences in the PK values between the healthy subjects and the severely decompensated subjects were much more pronounced such that peak tigecycline concentration was increased

to 25 %, clearance was decreased to 55 %, AUC was increased to 105 %, and there was a 97 % increase in the amount of drug that was excreted unchanged in the urine. Based on these pharmacokinetics, the authors suggested that patients with severely decompensated cirrhosis should be administered a tigecycline regimen consisting of a 100-mg loading dose followed by a reduced maintenance dose of 25 mg every 12 h. Simulation and modeling results suggest that this 50 % decrease in the maintenance dose would result in tigecycline AUCs that were similar to those observed in healthy subjects (Korth-Bradley et al. 2011).

Pharmacodynamics

In Vivo and In Vitro Assessment of Pharmacodynamics

Dose fractionation is an important method in determining antimicrobial activity. It involves evaluating the same total daily dose divided up into varying dosing frequencies. Here, researchers can determine the pharmacodynamic indices (AUC:MIC ratio, $\%T>\text{MIC}$, C_{max}:MIC ratio) for a particular drug in order to explore the exposure–response relationship that is most predictive of efficacy. This method was utilized in two neutropenic murine thigh model studies, both seeking to identify the pharmacodynamic index most indicative of tigecycline's antimicrobial activity (van Ogtrop et al. 2000; Crandon et al. 2009a). The study by van Ogtrop et al. postulated that tigecycline demonstrates time-dependent killing in the eradication of *Streptococcus pneumoniae*, *Escherichia coli*, and *Klebsiella pneumoniae*. However, when considering tigecycline's prolonged postantibiotic effect of 4.9 and 8.9 h against *E. coli* and *S. pneumoniae* respectively, combined with data showing a similar long half-life in humans, it appeared suggestive of tigecycline as an AUC:MIC ratio-driven drug. A subsequent neutropenic murine-thigh infection model conducted by Crandon et al. demonstrated that *f*AUC:MIC ratio was the PD index indicative of tigecycline efficacy when the protein binding of the agent is taken into consideration (Crandon et al. 2009a). Additionally, as mentioned in the previous edition to this book by (Andes and Craig 2007), the results of the study by van Ogtrop et al. changed once the ineffective 48-h dosing regimen was eliminated. When this regimen was removed, tigecycline efficacy was associated with the pharmacodynamic index identified in other studies, AUC:MIC ratio (Fig. 17.1). Several other murine studies have also come to the same conclusion that AUC:MIC ratio is the pharmacodynamic index that best describes the efficacy of tigecycline (Crandon et al. 2009a; Koomanachai et al. 2009a, b; Nicasio et al. 2009). In these studies, tigecycline was tested against *Staphylococcus aureus*, *K. pneumoniae*, *E. coli*, and *Acinetobacter baumannii* using murine-infection models and the resulting exposure response targets are highlighted in Table 17.1 (Crandon et al. 2009a; Koomanachai et al. 2009a, b; Nicasio et al. 2009). Additional evidence to support AUC:MIC ratio as the effective pharmacodynamic index include the sub-MIC effects exhibited by tigecycline in both an in vivo and time-kill assay. These sub-MIC effects contribute

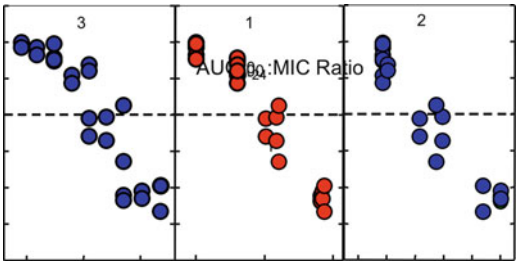

Fig. 17.1 Relationship between tigecycline efficacy and the pharmacodynamic–pharmacokinetic indices (*f*AUC:MIC ratio, *%fT*>MIC, fC_{max}:MIC ratio) in the neutropenic murine thigh infection model

Table 17.1 Pharmacodynamic exposure–response indices for $AUC_{0\text{-}24}$:MIC ratio in varying in vivo models

Organism (*n*)	MIC (mg/L)	Type of study	*f*AUC:MIC ratio exposure response target			Reference
			Stasis	EI_{50}	EI_{80}	
S. aureus (4)	0.125–0.5	Neutropenic murine thigh	15–20[a]	n/a	n/a	van Ogtrop et al. (2000)
E. coli (5)	0.125–0.5	Neutropenic murine thigh	5.1	4.5	7.3	Nicasio et al. (2009)
K. pneumoniae (6)	0.5–2	Immunocompetent murine thigh	1.2	1.3	1.8	Nicasio et al. (2009)
S. aureus (7)	0.125–0.5	Neutropenic murine thigh	2.8	2.6	5.4	Crandon et al. (2009a)
S. aureus (6)	0.125–0.5	Neutropenic murine pneumonia	1.9	1.8	3.0	Koomanachai et al. (2009a)
A. baumannii (5)	0.25–1	Murine pneumonia	5.9	8.2	17.2	Koomanachai et al. (2009b)

EI_{50} and EI_{80}: exposure index at 50 % and 80 %
[a]AUC:MIC ratio is based on total AUC:MIC ratio

to tigecycline's efficacy despite concentrations at or near 0 *%T*>MIC (Nicasio et al. 2009; Scheetz et al. 2007; Crandon et al. 2009a).

In each of the animal and in vitro studies, tigecycline has been depicted as a bacteriostatic drug based on its efficacy against *E. coli*, *K. pneumoniae*, and *S. aureus*. Interestingly, time-kill assay data has shown the activity of tigecycline against *Streptococcus pneumoniae* and *A. baumannii* to produce a ≥2.8 $\log_{10}$CFU/mL decrease in bacterial density, denoting activity that is concordant with a bactericidal definition (Hoellman et al. 2000; Scheetz et al. 2007). In these time-kill experiments, a couple of key findings were observed, with the first being that tigecycline initially exhibited bacteriostatic activity but gradually eclipsed near bactericidal activity as the study duration reached 24 h (Hoellman et al. 2000) or 48 h (Scheetz et al. 2007). Also, in both experiments, maximal effects were observed at concentrations between 0.8 and 1 mg/L at maximal doses of 8 mg/L (Hoellman et al. 2000) and 4 mg/L (Scheetz et al. 2007). Similar gradual kill effects were exhibited in another time-kill assay by Huang et al. (2010), when tigecycline was

tested against a variety of *S. aureus* isolates and the decreases in bacterial burden appeared to reach maximum at approximately 2.5 $\log_{10}$CFU/mL.

Similar changes in activity have been exhibited in two murine pneumonia models in which tigecycline, when tested against methicillin-resistant *S. aureus* and *A. baumannii*, produced similar bactericidal reductions as the time-kill assays described above (Koomanachai et al. 2009a, b; Pichardo et al. 2010). The reason for this increased bactericidal activity in the murine pneumonia model may possibly be due to tigecycline's significantly higher lung penetration and the subsequently higher concentrations that are exhibited with an established lung infection (Crandon et al. 2009b). Despite the higher concentrations and reduced bacterial densities that can be obtained in the murine lung infection model, this may not be indicative of any additional improved survival as indicated in the study by Pichardo and colleagues. In this study, tigecycline was compared to imipenem in a murine pneumonia model. Despite tigecycline exhibiting similar decreases in the bacterial burden of the lungs as imipenem, only imipenem significantly decreased mortality while tigecycline was comparable to the nonantibiotic cohort (Pichardo et al. 2010).

Clinical Pharmacodynamic Assessment Through Exposure–Response Analysis

Exposure–response analyses are typically determined by integrating data from preclinical (in vivo or in vitro) and clinical trials, microbiological and population pharmacokinetic modeling. Briefly, the pharmacokinetic/pharmacodynamic (PK/PD) index most predictive of efficacy (typically derived from animal data) is used as a predictor for the categorical data (microbiological, safety, or clinical response). Subsequently, the population pharmacokinetics data are used to calculate the individual patient steady-state parameters. The pharmacokinetic population models are either taken from previously constructed studies or constructed from Phase 1, 2 and 3 patient or subject pharmacokinetic data. This data is then normalized by the MIC data isolated from clinical trial patients. As previously mentioned in the section entitled "In Vivo and In Vitro Assessment of Pharmacodynamics," AUC:MIC ratio is the PK-PD index most predictive of efficacy for tigecycline. This information is then integrated with clinical (efficacy and safety) data and microbiological response data, which is pooled from multiple relevant clinical trials. With the use of statistical tools such as univariable or multivariable logistic regression and classification and regression tree-derived (CART) analyses, researchers are able to discern the appropriate pharmacodynamic parameter breakpoints (continuous data) needed for positive clinical and/or microbiological outcomes (categorical data) (Mouton et al. 2007).

Exposure–Response in Complicated Intra-abdominal Infection

In a 2006 study by van Wart and colleagues, a population pharmacokinetic model was generated from data taken from a Phase 2 and Phase 3 study. This data was composed of clinical data from patients who were diagnosed with complicated intra-abdominal

Table 17.2 Human exposure–response 24-h AUC:MIC ratio targets

Primary organisms	Study type	AUC:MIC ratio target	Reference
S. aureus; *Streptococcus* sp.; *Enterococcus* sp.	cSSSI	17.9	Meagher et al. (2007)
Enterobacteriaceae; *E. coli*	cIAI	6.94	Passarell et al. (2008)
Enterobacteriaceae	cIAI	3.1	Bhavnani et al. (2010)
S. pneumoniae	CAP	12.8 (free)-time to fever resolution	Rubino et al. (2012)
–	CAP	AUC = 6.87 mg h/L for occurrence of nausea and vomiting	Rubino et al. (2012)
Pseudomonas aeruginosa; *Acinetobacter* sp.; Enterobacteriaeace; *S. aureus*	HAP	3.56 (note: fAUC/MIC)	Bhavnani et al. (2012)

infection (cIAI) and complicated skin and skin structure infections (cSSSI), in addition to tigecycline PK concentrations. These data allowed for the identification of pharmacodynamic breakpoints associated with microbiological and clinical success in cIAI for a small cohort of patients infected with Enterobacteriaceae (Passarell et al. 2008). The study identified a CART-derived AUC:MIC ratio breakpoint of 6.96 for patients infected with Enterobacteriaceae pathogens. When this breakpoint was applied to Monte Carlo simulations, it was observed that tigecycline therapy was associated with >90 % microbiological response at an MIC of 0.25 mg/L (Ambrose et al. 2009).

More recently, the same group discerned the clinical efficacy and microbiological breakpoint of success in a larger populous of cIAI patients in order to understand the influence of various clinical factors on this success, alone or with drug exposures (Bhavnani et al. 2010). In this study, the data derived from the final multivariate logistic regression demonstrated six clinical factors that were predictive of clinical success: weight <94 kg; the absence of *P. aeruginosa* in the baseline cultures; an APACHE II score of <13; non-Hispanic race; complicated appendicitis or cholecystitis; and a ratio of AUC:MIC ratio ≥3.1 (Table 17.2). Interestingly, the study observed that clinical success was more closely associated with pathogens with the highest MIC values rather than to the type of bacterial species. Moreover, the study elucidated that the probability of clinical success was lower if the AUC:MIC ratio was <3.1, despite having all other identified factors set to favorable conditions. The average model-predicted probability of clinical success was even lower when ≥2 unfavorable factors were present (Bhavnani et al. 2010). Overall, this study showed that individual and multiple factors, including the AUC:MIC ratio, have large impacts on clinical success.

Exposure–Response in cSSSI

In the study by Meagher and colleagues, a similar exposure–response experimental approach was conducted as in the study by Passarell and colleagues (Table 17.2). Herein, using patient data that was pooled from a Phase 2 and two Phase 3 tigecycline

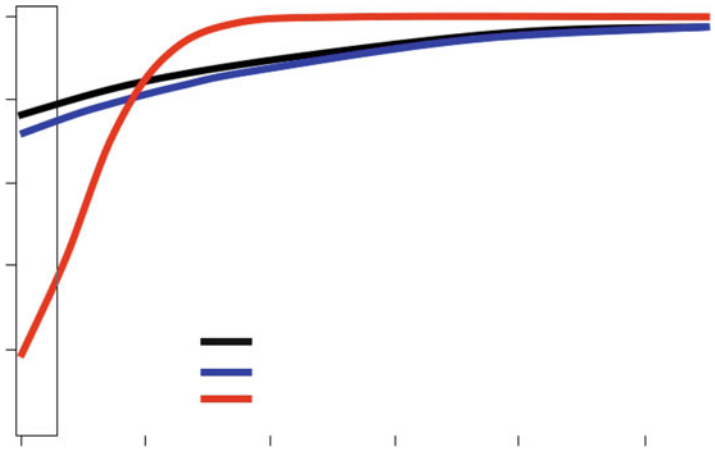

Fig. 17.2 The relationship between tigecycline probability of eradication and *f*AUC:MIC ratio versus Gram-positive and Gram-negative pathogens in patients with complicated skin and skin structure infections

cSSSI clinical studies, it was determined that the tigecycline breakpoint for clinical success and microbiological response is an AUC:MIC ratio breakpoint of 17.9 (Meagher et al. 2007) (Fig. 17.2, Table 17.2). When this breakpoint was utilized for Monte Carlo simulations, it was determined, as in the cIAI study, that tigecycline was able to capture an MIC of 0.25 mg/L with >90 % probability (Ambrose et al. 2009).

Exposure–Response in Pneumonia

In a most recent exposure response study (Bhavnani et al. 2012), it was reaffirmed that *f*AUC:MIC ratio is the pharmacodynamic index most predictive of tigecycline efficacy. This study examined patients with hospital acquired pneumonia (HAP) and following the same methodological approach as the other three previously mentioned exposure–response studies, patients were infected with at least one of the following bacterial organisms: Enterobacteriaceae species, *S. aureus*, *Acinetobacter* sp., and *Pseudomonas aeruginosa* (Meagher et al. 2007; Passarell et al. 2008; Bhavnani et al. 2010, 2012). Among the pharmacodynamic target indices observed, the *f*AUC:MIC ratio necessary for clinical success with HAP was 3.56 (Table 17.2). This lower pharmacodynamic index for HAP as compared to other infections sites (e.g., cSSSI or cIAI) is possibly a result of the comparably extensive tissue distribution of tigecycline (Bhavnani et al. 2012).

In another exposure–response study by the same group, exposure estimates were explored for community acquired pneumonia (CAP) (Rubino et al. 2012). In this study, patient data was obtained and pooled from two Phase 3 clinical trials for CAP. Much like the other pneumonia exposure–response study, population pharmacokinetic parameters were derived from the study by Rubino and colleagues and integrated with

pharmacokinetic data and baseline MIC values and then applied to microbiological and clinical evaluable patients. These MIC values were mainly for *S. pneumoniae*, as monomicrobial infections were evaluated. However, unlike the other exposure response studies, there did not appear to be a relationship between tigecycline exposures and efficacy at test of cure. Despite this, a CART-derived threshold exposure–response index of fAUC:MIC ratio ≥12.8 was observed to be associated with an improved time to defervescence (Table 17.2). Also noteworthy was the unveiling of an exposure safety response value as derived from CART analysis in the form of an AUC above a threshold value. More information is provided about exposure–safety response in the section below (Rubino et al. 2012).

Toxicodynamics

When focusing on safety and using multivariable logistic regression, the study by Rubino and colleagues uncovered that sex (female) and AUC (CART derived threshold of ≥6.87 mg h/L) were significant predictors of nausea and/or vomiting (Rubino et al. 2012). Other analysis on this topic have observed that a 1 unit increase in the 24 h AUC value is associated with an 18 % increased risk of nausea and vomiting (Passarell et al. 2005). Also, the CAP exposure–response study described that baseline bilirubin was also a significant variable; however, it was deemed inappropriate because of its lack of linearity with tigecycline exposures (Rubino et al. 2012) This may result in maximum tigecycline exposure changes leading to an insignificant increase in the bilirubin value after baseline.

Immunomodulatory Effects

As shown in a study by Salvatore et al. which employed a murine model, tigecycline has the potential to act as an immunomodulatory agent (Salvatore et al. 2009). This study showed that administration of tigecycline in a murine pneumonia model with *Mycobacterium pneumoniae* improved immunological and inflammatory effects in the lungs by significantly reducing pulmonary cytokine and chemokines and improving histological inflammation. These responses have been shown to be important factors for improving disease disparity linked with *Mycobacterium pneumoniae* pneumonia infections.

Conclusion

Tigecycline is a novel, first-in-class glycylcycline antimicrobial that has a wide range of antimicrobial activity. Due to its unique pharmacokinetic parameters, such as large volume of distribution and significant protein binding, this agent is a

preferred treatment option for infections involving tissue sites rather than bloodstream infections. Multiple pharmacodynamic studies have evaluated the best way to optimize the efficacy of this agent, with results showing that the PK–PD index most associated with efficacy is AUC:MIC ratio. Based upon the unique PK and PD of this agent, additional studies to further explore how best to optimize the dosing strategy of tigecycline are warranted.

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Index

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