

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

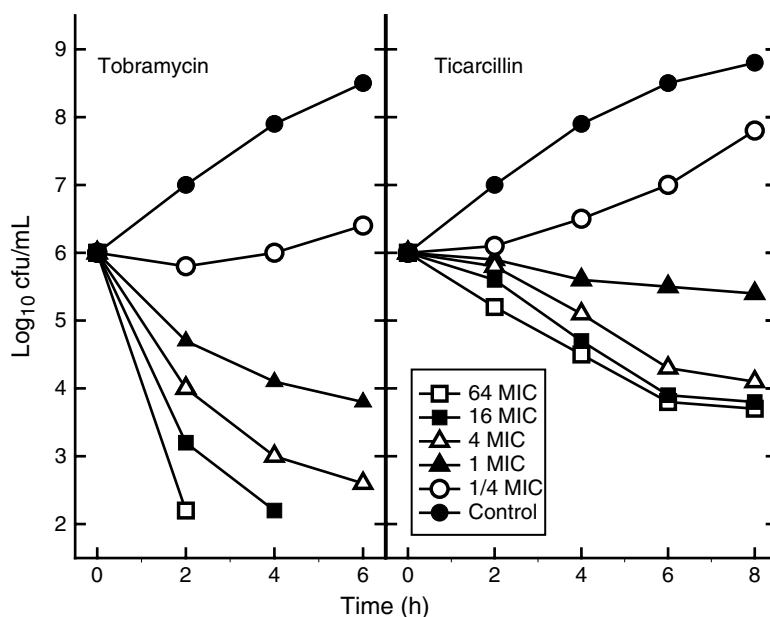


Fig. 2.1 Time–kill curves for *P. aeruginosa* ATCC 27853 with exposure to tobramycin and ticarcillin at concentrations from one-fourth to 64 times the MIC. Redrawn from Fig. 1 in Craig WA, Ebert SC. Killing and regrowth of bacteria in vitro: a review. *Scand J Infect Dis* 1991; Suppl 74:63–70

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

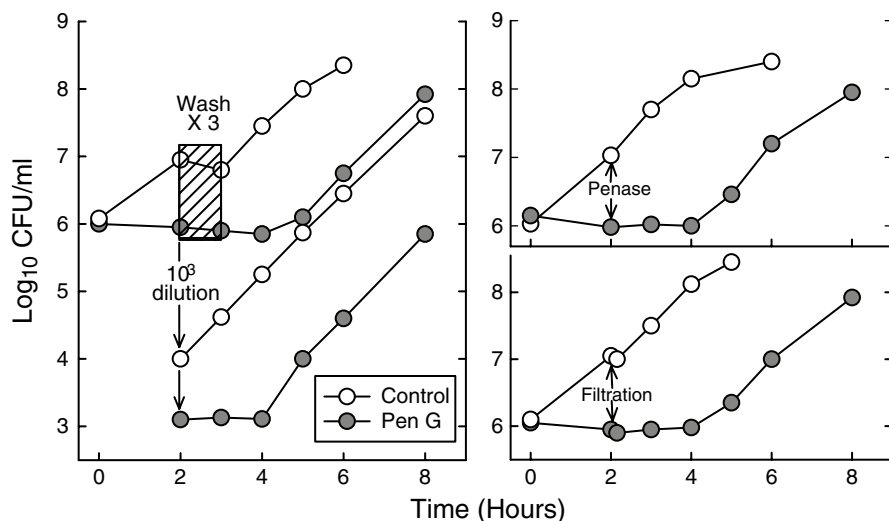


Fig. 2.2 Postantibiotic effects (PAEs) induced by a 2-h exposure of *S. aureus* ATCC 6538P in broth to 0.05 µg/ml of penicillin G using rapid drug removal by repeated washing, a 10⁻³ dilution, addition of penicillinase or filter filtration of the culture. Redrawn from Fig. 2.8.1 in Craig and Gudmundsson (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 10⁶ 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 10⁴ 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:

$$PAE = T - C \quad (2.1)$$

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-Törnqvist 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

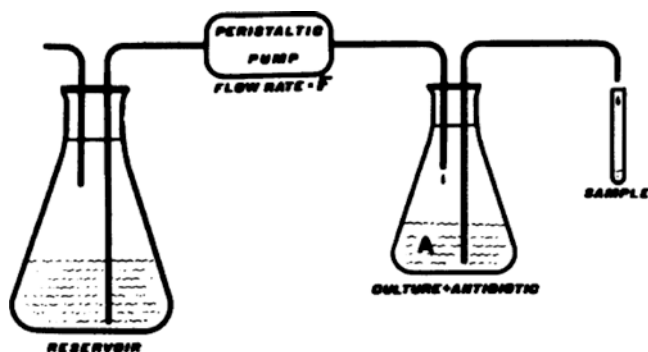


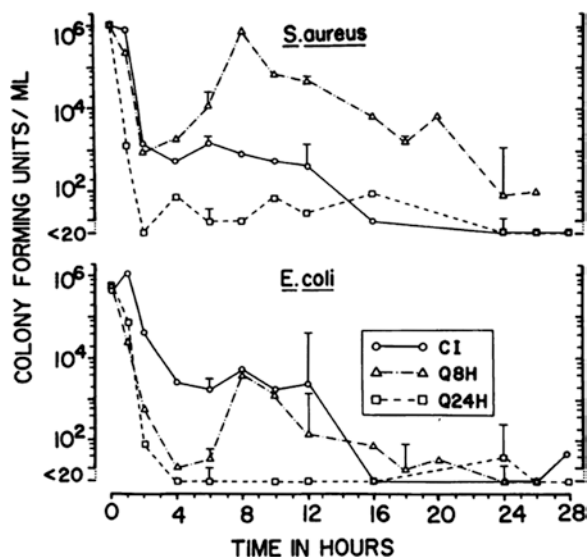
Fig. 2.3 Early dilution in vitro kinetic model. Republished with permission from Grasso et al. (1978)

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 extent 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 amount 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 10⁵–10⁶ CFU/ml. For fluoroquinolones antibiotics studies have not shown much difference in activity even up to an inoculum of 10⁹ 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 at 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 (Acired 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

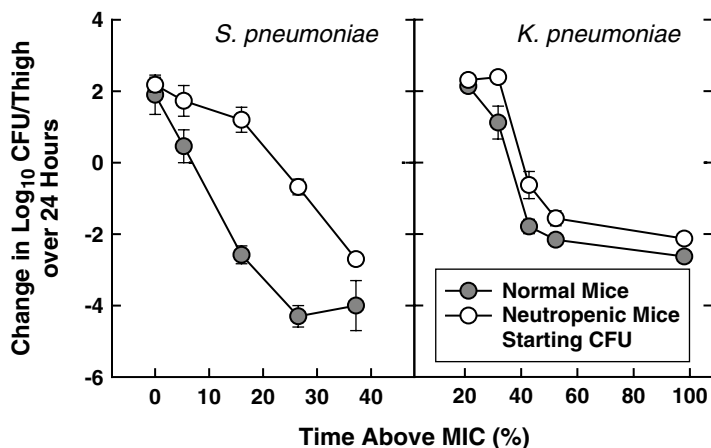
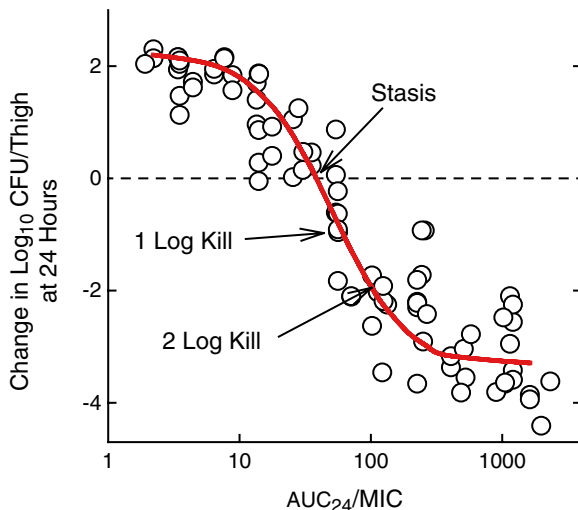


Fig. 2.5 Activity of ceftibiprole against *S. pneumoniae* ATCC 10813 (left panel) and *K. pneumoniae* ATCC 43816 (right panel) in the thighs of normal (non-neutropenic) and neutropenic mice. Republished with permission from Craig (2008)

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 log₁₀ 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 ceftibiprole against *S. pneumoniae* than *K. pneumoniae* (Craig and Andes 2008).

The starting inoculum can range from about 10⁵ to 10⁸ 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 10⁵ 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 10⁵ to 10⁷ (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)



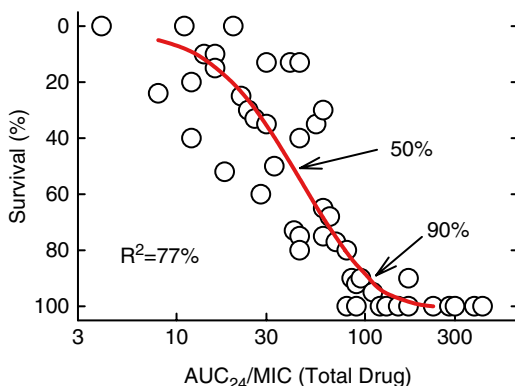
Usually the CFU/g or thigh is correlated with the serum kinetics of the drug. Studies with microdialysis have demonstrated in rats and humans that the concentration of drug in muscle interstitial fluid is very similar to the free drug concentration in serum (Kover et al. 1997; Liu et al. 2002). Figure 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. Nueremberger 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.

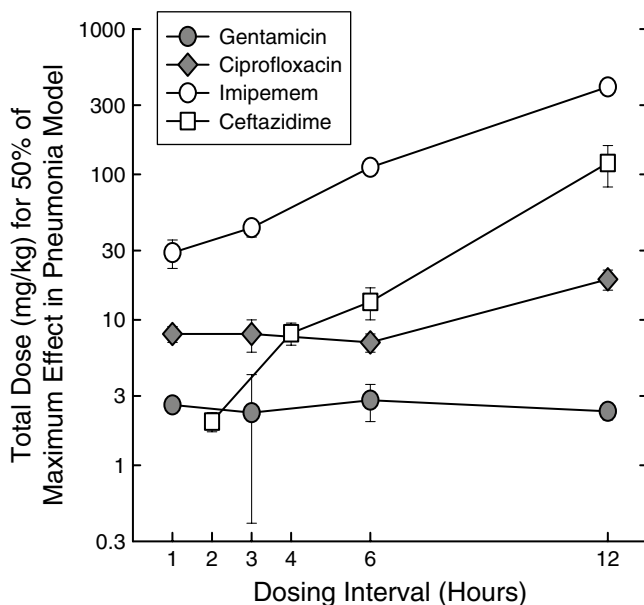


Fig. 2.8 Impact of the dosing interval for gentamicin, ciprofloxacin, imipenem, and ceftazidime on the 50 % effective dose (ED_{50}) against *K. pneumoniae* or *E. coli* in the lungs of neutropenic mice. Redrawn from data in Leggett et al. (1989, 1991)

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 intra-tracheal 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 ≤ 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.

References

- Acred P, Hunter PA, Mizen L, Rolinson GN (1970) Alpha-amino-para-hydroxybenzyl penicillin (BRL 2333), a new broad-spectrum semisynthetic penicillin: *in vivo* evaluation. *Antimicrob Agents Chemother* 1:416–422
- Acred P, Ryan DM, Sowa MA, Watts CM (1981) The in-vivo antibacterial activity of ceftazidime (Gr 20263) – a comparison with other new beta-lactam antibiotics and gentamicin. *J Antimicrob Chemother* 8(Suppl B):247–255
- Ahmed A, Paris MM, Trujillo M, Hickey SM, Wubbel L, Shelton SL, McCracken GJ Jr (1997) Once-daily gentamicin therapy for experimental *Escherichia coli* meningitis. *Antimicrob Agents Chemother* 41:49–53
- Andes D, Craig WA (1998a) In vivo activities of amoxicillin and amoxicillin-clavulanate against *Streptococcus pneumoniae*: application to breakpoint determinations. *Antimicrob Agents Chemother* 42:2375–2379
- Andes DR, Craig WA (1998b) Pharmacodynamics of fluoroquinolones in experimental models of endocarditis. *Clin Infect Dis* 27:47–50
- Andes DR, Craig WA (1999) Pharmacokinetics and pharmacodynamics of antibiotics in meningitis. *Infect Dis Clin N Am* 13:595–618
- Andes DR, Craig WA (2002) Animal model pharmacokinetics and pharmacodynamics: a critical review. *Int J Antimicrob Agents* 19:261–268
- Andes D, Craig WA (2005) Treatment of infections with ESBL-producing organisms: pharmacokinetic and pharmacodynamic considerations. *Clin Microbiol Infect* 11(Suppl 6):10–17
- Andes A, Craig WA (2007) In vivo pharmacodynamic activity of the glycopeptide dalbavancin. *Antimicrob Agents Chemother* 51:1633–1642
- Azoulay-Dupuis E, Bedos JP, Vallee E, Hardy DJ, Swanson RN, Pocidalo JJ (1991a) Antipneumococcal activity of ciprofloxacin, ofloxacin, and temafloxacin in an experimental mouse pneumonia model at various stages of the disease. *J Infect Dis* 163:319–324
- Azoulay-Dupuis E, Bedos JP, Vallee E, Pocidalo JJ (1991b) Comparative activity of fluorinated quinolones in acute and subacute *Streptococcus pneumoniae* pneumonia models: efficacy of temafloxacin. *J Antimicrob Chemother* 28:45–53
- Bakker-Woudenberg IAJM, de Jong-Hoederop JY, Michel MF (1979) Efficacy of antimicrobial chemotherapy in experimental rat pneumonia: effects of impaired phagocytosis. *Infect Immun* 25:366–375
- Bakker-Woudenberg IA, ten Kate MT, Goessens WH, Mouton JW (2006) Effect of treatment duration on pharmacokinetic/pharmacodynamic indices correlating with therapeutic efficacy of ceftazidime in experimental *Klebsiella pneumoniae* lung infection. *Antimicrob Agents Chemother* 50:2919–2925

- Bergan T, Carlsen IB, Fuglesang JE (1980) An in vitro model for monitoring bacterial responses to antibiotic agents under simulated in vivo conditions. *Infection* 8:S103–S108
- Blaser J (1985) In-vitro model for simultaneous simulation of the serum kinetics of two drugs with different half-lives. *J Antimicrob Chemother* 15(Suppl A):125–130
- Blaser J, Stone BB, Groner MC, Zinner SH (1987) Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. *Antimicrob Agents Chemother* 31:1054–1060
- Bonapace CR, Friedrich LV, Bosso JA, White RL (2002) Determination of antibiotic effect in an in vitro pharmacodynamic model: comparison with an established animal model of infection. *Antimicrob Agents Chemother* 46:3574–3579
- Boutoille D, Jacqueline C, Le Mabecque V, Potel G, Caillon J (2009) In vivo impact of the MexAB-OprM efflux system on beta-lactam efficacy in an experimental model of *Pseudomonas aeruginosa* infection. *Int J Antimicrob Agents* 33:417–420
- Craig WA, Andes DR (2008) In vivo pharmacodynamics of ceftobiprole against multiple bacterial pathogens in murine thigh- and lung-infection models. *Antimicrob Agents Chemother* 52:3492–3496
- Craig WA, Gudmundsson S (1996) Postantibiotic effect (Chapter 8). In: Lorian V (ed) *Antibiotics in laboratory medicine*, 4th edn. Williams and Wilkins, Baltimore, MD, pp 296–329
- Crandon JL, Kuti JL, Nicolau DP (2010) Comparative efficacies of human simulated exposures of telavancin and vancomycin against methicillin resistant *Staphylococcus aureus* with a range of vancomycin MICs in a murine pneumonia model. *Antimicrob Agents Chemother* 54:5115–5119
- Davis SD (1975) Activity of gentamicin, tobramycin, polymyxin B, and colistimethate in mouse protection tests with *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 8:50–53
- den Hollander JG, Fuursted K, Verbrugh HA, Mouton JW (1998) Duration and clinical relevance of postantibiotic effect in relation to the dosing interval. *Antimicrob Agents Chemother* 42:749–754
- Drusano GL, Fregeau C, Liu W, Brown DL, Louie A (2010) Impact of burden of granulocyte clearance of bacteria in a mouse thigh infection model. *Antimicrob Agents Chemother* 54:4368–4372
- Drusano GL, Johnson DE, Rosen M, Standiford HC (1993) Pharmacodynamics of a fluoroquinolones antimicrobial agent in a neutropenic rat model of pseudomonas sepsis. *Antimicrob Agents Chemother* 37:483–490
- Dudley MN, Blaser J, Gilbert D, Zinner SH (1990) Significance of “extravascular” protein binding for antimicrobial pharmacodynamics in an in vitro capillary model of infection. *Antimicrob Agents Chemother* 34:98–101
- Eagle H, Fleishman R, Musselman AD (1950) Effect of schedule of administration on the therapeutic efficacy of penicillin. *Am J Med* 9:280–299
- Firsov AA, Vostrov SN, Kononenko OV, Zinner SH, Portnov YA (1999) Prediction of the effects of inoculum size on the antimicrobial action of trovafloxacin and ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli* in an in vitro dynamic model. *Antimicrob Agents Chemother* 43:498–502
- Firsov AA, Vostrov SN, Lubenko IY, Drlica K, Portnoy YA, Zinner SH (2003) In vitro pharmacodynamic evaluation of the mutant selection window hypothesis using four fluoroquinolones against *Staphylococcus aureus*. *Antimicrob Agents Chemother* 47:1604–1613
- Frimodt-Moller N, Bentzon MW, Thompson VF (1986) Experimental infection with *Streptococcus pneumoniae* in mice: correlation of in vitro and pharmacokinetic parameters with in vivo effect for 14 cephalosporins. *J Infect Dis* 154:511–517
- Garrett ER, Miller GH (1965) Kinetics and mechanism of action of antibiotics on microorganisms. 3: inhibitory action of tetracycline and chloramphenicol on *Escherichia coli* established by total and viable counts. *J Pharm Sci* 54:427–431
- Garrison MW, Vance-Bryan K, Larson TA, Toscano JP, Rotschaefer JC (1990) Assessment of the effects of protein binding on daptomycin and vancomycin killing of *Staphylococcus aureus* by using an in vitro pharmacodynamic model. *Antimicrob Agents Chemother* 34:1925–1931
- Garrod LP (1948) The bactericidal action of streptomycin. *Br Med J* 1:382–386

- Gavalda J, Lopez P, Martin T, Gomis X, Ramirez JL, Azuaje C, Almirante B, Pahissa A (2002) Efficacy of ceftriaxone and gentamicin given once a day by using human-like pharmacokinetics in treatment of experimental staphylococcal endocarditis. *Antimicrob Agents Chemother* 46:378–384
- Gerber AU, Craig WA (1981) Growth kinetics of respiratory pathogens after short exposures to ampicillin and erythromycin *in vitro*. *J Antimicrob Chemother* 8(Suppl B):81–91
- Gerber AU, Vastola AP, Brandel J, Craig WA (1982) Selection of aminoglycoside-resistant variants of *Pseudomonas aeruginosa* in an *in vivo* model. *J Infect Dis* 146:691–697
- Gerber AU, Craig WA, Brugger H-P, Feller C, Vastola AP, Brandel J (1983) Impact of dosing intervals on activity of gentamicin and ticarcillin against *Pseudomonas aeruginosa* in granulocytic mice. *J Infect Dis* 147:910–917
- Grasso S, Meinardi G, de Carneri I, Tamassia V (1978) New *in vitro* model to study the effect of antibiotic concentration and rate of elimination on antibacterial activity. *Antimicrob Agents Chemother* 13:570–576
- Hanberger H, Nilsson LE, Kihlstrom E, Maller R (1990) Postantibiotic effect of β -lactam antibiotics on *Escherichia coli* evaluated by bioluminescence assay of bacterial ATP. *Antimicrob Agents Chemother* 34:102–106
- Hulten K, Rigo R, Gustafsson I, Engstrand L (1996) New pharmacokinetic *in vitro* model for studies of antibiotic activity against intracellular microorganisms. *Antimicrob Agents Chemother* 40:2727–2731
- Hunter PA, Rolinson GN, Witting DA (1973) Comparative activity of amoxicillin and ampicillin in experimental infection in mice. *Antimicrob Agents Chemother* 4:285–293
- Kang SL, Rybak MJ (1995) Pharmacodynamics of RP 59500 alone and in combination with vancomycin against *Staphylococcus aureus* in an *in vitro*-infected fibrin clot model. *Antimicrob Agents Chemother* 39:1505–1511
- Keil S, Weidemann B (1995) Mathematical corrections for bacterial loss in pharmacodynamic *in vitro* dilution models. *Antimicrob Agents Chemother* 39:1054–1058
- Kim A, Banevicius MA, Nicolau DP (2008) *In vivo* pharmacodynamic profiling of doripenem against *Pseudomonas aeruginosa* by simulating human exposures. *Antimicrob Agents Chemother* 52:2497–2502
- Knudsen JD, Fuusted K, Raber S, Espersen F, Frimodt-Moller N (2000) Pharmacodynamics of glycopeptides in the mouse peritonitis model of *Streptococcus pneumoniae* and *Staphylococcus aureus* infections. *Antimicrob Agents Chemother* 44:1247–1254
- Knudsen JD, Odenholt I, Erlendsdottir H, Gottfredsson M, Cars O, Frimodt-Moller N, Espersen F, Kristinsson KG, Gudmundsson S (2003) Selection of resistant *Streptococcus pneumoniae* during penicillin treatment *in vitro* and in three animal models. *Antimicrob Agents Chemother* 47:2499–2506
- Koomanachal P, Crandon JL, Banevicius MA, Peng L, Nicolau DP (2009) Pharmacodynamic profile of tigecycline against methicillin-resistant *Staphylococcus aureus* in an experimental pneumonia model. *Antimicrob Agents Chemother* 53:5060–5063
- Kover A, Dalla Costa T, Derendorf H (1997) Comparison of plasma and free tissue levels of ceftriaxone in rats by microdialysis. *J Pharm Sci* 86:52–56
- Kunst WM, Mattie H (1978) Cefazolin and cephradine relationship between antibacterial activity *in vitro* and in mice experimentally infected with *Escherichia coli*. *J Infect Dis* 137:391–401
- Ledergerber B, Blaser J, Luthy R (1985) Computer-controlled *in vitro* simulation of multiple dosing regimens. *J Antimicrob Chemother* 15(Suppl A):169–173
- Lee D-G, Murakami Y, Andes DR, Craig WA (2013) Inoculum effect of ceftibiprole, daptomycin, linezolid and vancomycin with *Staphylococcus aureus* and *Streptococcus pneumoniae* in neutropenic mice at 10^5 and 10^7 CFU in opposite thighs. *Antimicrob Agents Chemother* 57(3):1434–1441
- Leggett JE, Fantin B, Ebert S, Totsuka K, Vogelmann B, Calame W, Mattie H, Craig WA (1989) Comparative antibiotic dose-effect relations at several dosing intervals in murine pneumonitis and thigh-infection models. *J Infect Dis* 159:281–292
- Leggett JE, Ebert S, Fantin B, Craig WA (1991) Comparative dose-effect relations at several dosing intervals for beta-lactam, aminoglycoside and quinolone antibiotics against gram-negative bacilli in murine thigh-infection and pneumonitis models. *Scand J Infect Dis* 74(Suppl):179–184

- Liu P, Muller M, Grant M, Webb AI, Obermann B, Derendorf H (2002) Interstitial tissue concentrations of cefpodoxime. *J Antimicrob Chemother* 50(Suppl):19–22
- Lorian V, Zak O, Sutter J, Bruecher C (1985) Staphylococci in vitro and in vivo. *Diag Microbiol Infect Dis* 3:433–444
- Lorian V, Ernst J, Amaral L (1989) The post-antibiotic effect defined by bacterial morphology. *J Antimicrob Chemother* 23:485–491
- Louie A, Vanscoy BD, Heine HS III, Liu W, Abshire T, Holman K, Kulawy R, Brown DL, Drusano GL (2012) Differential effects of linezolid and ciprofloxacin on toxin production by *Bacillus anthracis* in an in vitro pharmacodynamic system. *Antimicrob Agents Chemother* 56:513–517
- Lowdin E, Odenholt I, Cars O (1998) In vitro studies of pharmacodynamic properties of vancomycin and against *Staphylococcus aureus* and *Staphylococcus epidermitis*. *Antimicrob Agents Chemother* 42:2739–2744
- Lutsar I, Ahmed A, Friedland IR, Trujillo M, Wubbel L, Olsen K, McCracken GH Jr (1997) Pharmacodynamics and bactericidal activity of ceftriaxone therapy in experimental cephalosporin-resistant pneumococcal meningitis. *Antimicrob Agents Chemother* 41:2414–2417
- Lutsar I, Friedland IR, Wubbel L, McCoig CC, Jafri HS, Ng W, Ghaffar F, McCracken GH Jr (1998) Pharmacodynamics of gatifloxacin in cerebrospinal fluid in experimental cephalosporin-resistant pneumococcal meningitis. *Antimicrob Agents Chemother* 42:2650–2655
- MacKenzie FM, Gould IM, Chapman DG, Jason D (1994) Comparison of methodologies used in assessing the postantibiotic effect. *J Antimicrob Chemother* 34:223–230
- Maglio D, Ong C, Barevicius GQ, Nightingale CH, Nicolau DP (2007) Determination of the in vivo profile of cefepime against extended-spectrum- β -lactamase-producing *Escherichia coli* at various inocula. *Antimicrob Agents Chemother* 48:1941–1947
- McDonald PJ, Wetherall BL, Pruul H (1981) Postantibiotic leukocyte enhancement: increased susceptibility of bacteria pretreated with antibiotics to activity of leukocytes. *Rev Infect Dis* 3:38–44
- Mouton JW, den Hollander JG (1994) Killing of *Pseudomonas aeruginosa* during continuous and intermittent infusion of ceftazidime in an in vitro pharmacokinetic model. *Antimicrob Agents Chemother* 38:931–936
- Nicolau DP, Onyeji CO, Zhong M, Tessier PR, Banevicius MA, Nightingale CH (2000) Pharmacodynamic assessment of cefprozil against *Streptococcus pneumoniae*: implications for breakpoint determinations. *Antimicrob Agents Chemother* 44:1291–1295
- Noel AR, Bowker KE, MacGowan AP (2005) Pharmacodynamics of moxifloxacin against anaerobes studies in an in vitro pharmacokinetic model. *Antimicrob Agents Chemother* 49:4234–4239
- Nuernberger E, Helke K, Bishai WR (2005) Low-dose aerosol model of pneumococcal pneumonia in the mouse model: utility for evaluation of antimicrobial efficacy. *Int J Antimicrob Agents* 26:497–503
- Odenholt I, Holm SE, Cars O (1989) Effects of benzylpenicillin on *Streptococcus pyogenes* during the postantibiotic phase in vitro. *J Antimicrob Chemother* 24:147–156
- Odenholt I, Lowdin E, Cars O (2007) Pharmacodynamic effects of telavancin against methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains in the presence of human albumin or serum and in an in vitro kinetic model. *Antimicrob Agents Chemother* 51:3311–3316
- Odenholt-Tornqvist I, Lowdin E, Cars O (1992) Postantibiotic sub-MIC effects of vancomycin, roxithromycin, sparflaxacin, and amikacin. *Antimicrob Agents Chemother* 36:1852–1858
- Pennington JE, Stone RM (1979) Comparison of antibiotic regimens for treatment of experimental pneumonia due to pseudomonas. *J Infect Dis* 140:881–889
- Reeves DS (1985) Advantages and disadvantages of an in-vitro model with two compartments connected by a dialyzer: results of experiments with ciprofloxacin. *J Antimicrob Chemother* 15(Suppl A):159–167
- Roosendaal R, Bakker-Woudenberg IA, van den Berghe-van Raffe M, Vink-van den Berg JC, Michel BM (1989) Impact of the dosage schedule on the efficacy of ceftazidime, gentamicin and ciprofloxacin in Klebsiella pneumonia and septicemia in leukopenic rats. *Eur J Clin Microbiol Infect Dis* 10:878–887

- Roosendaal R, Bakker-Woudenberg IA, van den Berghe-van Raffe M, Michel MF (1986) Continuous versus intermittent administration of ceftazidime in experimental *Klebsiella pneumoniae* pneumonia in normal and leukopenic rats. *Antimicrob Agents Chemother* 30:403–408
- Sanberg A, Jensen KS, Baudoux P, Van Bambeke F, Tulkens PM, Frimodt-Moller N (2010) Intra- and extracellular activity of linezolid against *Staphylococcus aureus* in vitro and in vivo. *J Antimicrob Chemother* 65:962–973
- Schmidt LH, Walley A (1951) The influence of the dosage regimen on the therapeutic effectiveness of penicillin in experimental lobar pneumonia. *J Pharmacol Exp Ther* 103:479–488
- Schmidt LH, Walley A, Larson RD (1949) The influence of the dosing regimen on the therapeutic activity of penicillin G. *J Pharmacol Exp Ther* 96:258–268
- Scoriano F, Ponte C, Nieto E, Parra A (1996) Correlation on *in vitro* activity and pharmacokinetics parameters with *in vivo* effect of amoxycillin, co-amoxiclav and cefotaxime in a murine model of pneumococcal pneumonia. *J Antimicrob Chemother* 38:227–236
- Selbie FR (1954) Comparison of sodium and procaine penicillin in treatment of experimental staphylococcal infection in mice. *BMJ* 1:1350–1351
- Selbie FR, Simon RD (1952) Virulence of mice to *Staphylococcus pyogenes*: its measurement and its relation to certain *in vitro* properties. *Br J Exp Pathol* 33:315–326
- Shah PM, Junghanns W, Stille W (1976) Dosis-Wirkungs-Beziehung der Bakterizidie bei *E. coli*, *K. pneumoniae* und *Staphylococcus aureus*. *Dtsch Med Wochenschr* 101:325–328
- Smimova MV, Vostrov SN, Strukova EV, Dovzhenko SA, Kobrin MB, Portnoy YA, Zinner SH, Firsov AA (2009) The impact of duration of antibiotic exposure on bacterial resistance predictions using *in vitro* dynamic models. *J Antimicrob Chemother* 64:815–820
- Stearne LE, Gyssens IC, Goessens WH, Mouton JW, Oyen WJ, van der Meer JW, Verbrugh HA (2001) *In vivo* efficacy of trovafloxacin against *Bacteroides fragilis* in a mixed infection with either *Escherichia coli* or a vancomycin-resistant strain of *Enterococcus faecium* in an established abscess murine model. *Antimicrob Agents Chemother* 45:1394–1401
- Stearne LE, Goessens WH, Mouton JW, Gyssens IC (2007) Effect of dosing and dosing frequency on the efficacy of ceftizoxime and the emergence of ceftizoxime resistance during the early development of murine abscesses caused by *Bacteroides fragilis* and *Enterobacter cloacae* mixed infection. *Antimicrob Agents Chemother* 51:3605–3611
- Tam VH, Ledesma KR, Chang KT, Wang TY, Quinn JP (2009) Killing of *Escherichia coli* by beta-lactams at different inocula. *Diagn Microbiol Infect Dis* 64:166–171
- Tano E, Cars O, Lowdin E (2005) Pharmacodynamic studies of moxifloxacin and erythromycin against intracellular *Legionella pneumophila* in an *in vivo* kinetic model. *J Antimicrob Chemother* 56:240–242
- Tateda K, Takashima K, Miyazaki H, Matsumoto T, Hatori T, Yamaguchi K (1996) Noncompromized penicillin-resistant pneumococcal pneumonia CBA/J mouse model and comparative efficacies of antibiotics in this model. *Antimicrob Agents Chemother* 40:1520–1525
- Tauber MG, Doroshow CA, Hackbarth CJ, Rusnak MG, Drake TA, Sande MA (1984a) Antibacterial activity of beta-lactam antibiotics in experimental meningitis due to *Streptococcus pneumoniae*. *J Infect Dis* 149:568–574
- Tauber MG, Zak O, Scheld WM, Hengstler B, Sande MA (1984b) The postantibiotic effect in the treatment of experimental meningitis caused by *Streptococcus pneumoniae* in rabbits. *J Infect Dis* 149:575–583
- Toothaker RD, Welling PG, Craig WA (1982) An *in vitro* model for the study of antibacterial dosage regimen design. *J Pharm Sci* 71:861–864
- Tsaganos T, Skiadas I, Koutoukas P, Adamis T, Baxevanos N, Tzepe I, Pelekanou A, Giamarellos-Bouboulis EJ, Giamarellou H, Kanellakoulou K (2008) Efficacy and pharmacodynamics of linezolid, alone and in combination with rifampicin, in an experimental model of methicillin-resistant *Staphylococcus aureus* endocarditis. *J Antimicrob Chemother* 62:381–383
- Unadkat JD, Bartha F, Sheiner LB (1986) Simultaneous modeling of pharmacokinetic and pharmacodynamic with nonparametric kinetic and dynamic models. *Clin Pharmacol Ther* 40:86–93

- van't Wout JW, Linde I, Leijh PC, van Furth R (1989) Effect of radiation, cyclophosphamide, and etoposide (VP-16) on number of peripheral blood and peritoneal leukocytes in mice under normal conditions and during acute inflammatory reaction. *Inflammation* 13:1–14
- Vogelman B, Craig WA (1986) Kinetics of antimicrobial activity. *J Pediatr* 108:835–840
- Vogelman B, Gudmundsson S, Leggett J, Turnidge J, Ebert S, Craig WA (1988a) Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. *J Infect Dis* 158:831–847
- Vogelman B, Gudmundsson S, Turnidge J, Craig WA (1988b) The *in vivo* postantibiotic effect in a thigh infection in neutropenic mice. *J Infect Dis* 175:287–298
- Woodnut G, Berry V (1999) Two pharmacodynamic models for assessing the efficacy of amoxicillin-clavulanate against experimental respiratory tract infections caused by strains of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 43:29–34
- Wright DH, Gunderson BW, Hovde LB, Ross GH, Ibrahim KH, Rotschafer JC (2002) Comparative pharmacodynamics of three newer fluoroquinolones versus six strains of staphylococci in an in vitro model under aerobic and anaerobic conditions. *Antimicrob Agents Chemother* 46:1561–1563
- Zinner SH, Husson M, Klastersky J (1981) An artificial capillary in vitro kinetic model of antibiotic bactericidal activity. *J Infect Dis* 144:583–587
- Zuluaga AF, Salazar BE, Rodriguez CA, Zapata AX, Agudelo M, Vesga O (2006) Neutropenia induced in outbred mice by a simplified low-dose cyclophosphamide regimen: characterization and applicability to diverse experimental models of infectious diseases. *BMC Infect Dis* 6:55

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