

Animal model pharmacokinetics and pharmacodynamics: a critical review

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Abstract

Animals have been extensively used in the evaluation of antimicrobials. The value of animals in the pharmacokinetic and pharmacodynamic characterization of antimicrobials is critically reviewed. Animal studies have demonstrated that the pharmacokinetic/pharmacodynamic (PK/PD) target determining efficacy can vary for different classes of antimicrobials. However, the magnitude of the target required for bacteriological efficacy is relatively similar for various sites of infection, various pathogens and various drugs within the same class, provided free drug levels are used. © 2002 Elsevier Science B.V. and International Society of Chemotherapy. All rights reserved.

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1. Introduction

There are a large variety of animal models that provide investigators with the ability to characterize the pharmacokinetics and pharmacodynamics of antimicrobial therapy. These *in vivo* models can detail the time course relationship between serum and infection site antimicrobial concentrations. These measurements can then be correlated with the time course of antimicrobial activity at the site of infection. Modeling of the relationship between drug concentration and efficacy can allow one to determine which pharmacokinetic/pharmacodynamic (PK/PD) dosing parameter best correlates with treatment outcomes. While there may be pharmacokinetic, immune-state and infection site differences among various animal models, appropriate pharmacodynamic analyses most often account for these differences and allow comparison of data among studies possible. For example, by correcting for inter-species pharmacokinetic differences these studies can determine the magnitude of the PK/PD index necessary for antimicrobial efficacy across animal species, including humans. These analyses have been shown to be predictive of therapeutic success and failure against resis-

tant microorganisms in clinical trials. The ability to provide a PK/PD index magnitude target for clinical dosing has been particularly valuable for predicting outcomes against pathogens not encountered frequently enough in clinical trials to come to accurate conclusions.

1.1. Pharmacokinetics

Animal models have been very useful in describing the relationships between tissue concentrations and those in serum or plasma. The most common way of measuring tissue concentrations is from tissue homogenates [1]. However, tissues consist of two separate compartments that are mixed together when tissue homogenates are produced. The interstitial compartment and the intracellular compartment are distinct sites, and drugs often vary in their distribution in these two compartments. For example, beta-lactams provide high levels in interstitial fluids that are similar to those in serum, but very low to undetectable levels in intracellular fluid [1]. On the other hand, fluoroquinolones produce low interstitial and serum concentrations, but very high levels in intracellular fluid [2]. Since the intracellular compartment is usually of larger volume than interstitial fluids, tissue homogenates give lower levels than serum for beta-lactams and much higher levels than serum for macrolides. The important variable is the location of the pathogen, which for most bacteria is the extracellular

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space or interstitial fluids. Thus, for most pathogens the serum concentrations provide a good reflection of the interstitial drug levels and can be used for prediction of pharmacokinetics at the site of action.

Serum protein binding is another pharmacokinetic factor that can impact antimicrobial efficacy. There are numerous studies that have demonstrated that only the free or unbound fraction of drug is available for antimicrobial activity [3]. Studies by Merrikin, Briant and Rolinson demonstrated that the *in vivo* activity of a group of penicillins with similar pharmacokinetics and minimal inhibitory concentrations (MICs) against *Staphylococcus aureus* were directly related to the concentration of free drug [4].

While protein binding has little effect on the half-life of drugs eliminated by tubular secretion, it can markedly slow the elimination of drugs cleared predominantly by glomerular filtration [1,2]. If the activity of the drug is dependent on the duration of time concentrations exceed the MIC, a longer half-life may be beneficial. Thus, the overall impact of protein binding on antimicrobial activity is dependent on the type of drug and its route of elimination. Nevertheless, free drug concentrations must be considered when examining the relationship between pharmacokinetic parameters and *in vivo* activity.

One of the major differences in pharmacokinetics between animals and humans is that the rate of drug elimination is faster in animals [5–8]. This is especially true in small rodents, which are commonly used in animal infection models. Table 1 compares the half-life of drugs from six antimicrobial classes in mice with those in humans. In general, the half-life is 6- to 9-fold longer in humans than in mice. This difference can also have significant impact on treatment outcome in animal infection models. For example, once-daily dosing of aminoglycosides has been effective only in non-neutropenic medium sized animals [9]. Daily dosing of aminoglycosides has consistently been less effective than multiple daily administrations in small rodents infected with Enterobacteriaceae or neutropenic rodents infected with *Pseudomonas aeruginosa* [10,11].

Table 1
Serum elimination half-lives of selected antibiotics in mice and humans

Drug	Half-life in minutes	
	Mice	Humans
Amikacin	17	104
PCN	5	30
Imipenem	8	60
Cefazolin	15	108
Ciprofloxacin	32	240
Minocycline	120	1080

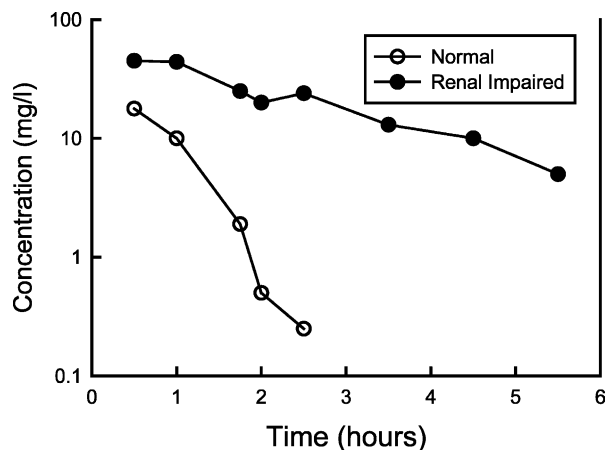


Fig. 1. Pharmacokinetics of amikacin at 30 mg/kg in mice with normal and impaired renal function.

The half-life of drugs eliminated primarily by the kidney can be lengthened in rodents by producing transient acute tubular injury with uranyl nitrate [12]. Fig. 1 shows the effect of uranyl nitrate-induced renal impairment on the pharmacokinetics of amikacin in mice [5]. The half-life of amikacin was increased from 18 to 93 min, which is much closer to the half-life observed in humans. This longer half-life can also result in enhanced efficacy in animal infection models. Fig. 2 shows the *in vivo* impact of different dosing frequencies on the amount of amikacin required to produce a net bacteriostatic effect over 24 h. In the left panel, neutropenic mice with normal renal function and a rapid half-life for amikacin required higher doses as the dosing interval was shifted from 6 to 24 h. *P. aeruginosa* was the only organism for which the increase in dose with 6- to 24-h dosing was relatively small. On the other hand, the left panel shows that the dose required for a bacteriostatic effect was lower and unaltered by the dosing interval when the mice had renal impairment and a longer half-life for amikacin.

1.2. Time-course of antimicrobial activity *in vivo*

Traditional *in vitro* measurements such as the MIC are used to predict outcome of antimicrobial therapy. While these measurements are a good indication of the potency of an antimicrobial they do not provide the type of information necessary to determine the optimal drug dose or dosing interval. The MIC test provides information about a drug concentration at a single time point. This tells us nothing about the effect of varying drug concentrations over time or whether there may be microbiological effects that persist after drug exposure (postantibiotic effects). Both *in vitro* and animal studies have been used to determine the impact of drug concentration on the rate and extent of antimicrobial killing. For example, aminoglycosides, fluoroquinolones

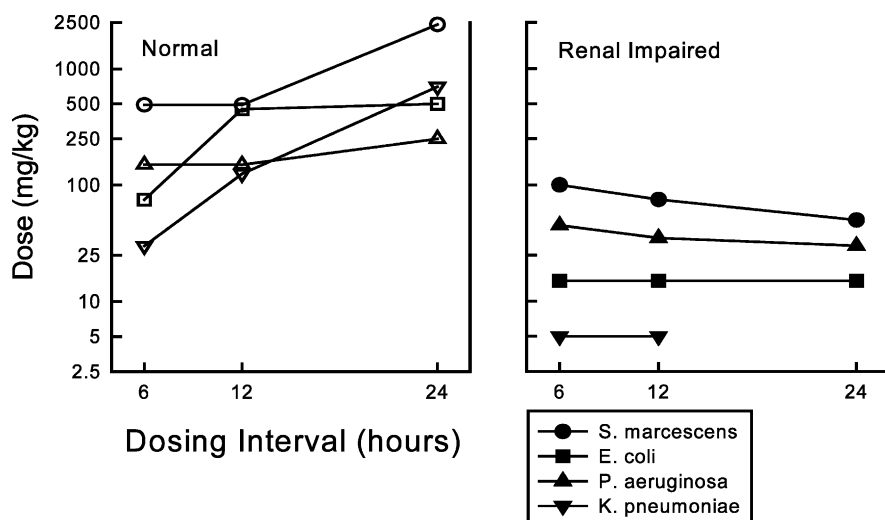


Fig. 2. The dose (mg/kg) of amikacin required to produce a bacteriostatic effect over 24 h in the thighs of neutropenic mice with normal renal function (left panel) and impaired renal function (right panel).

and ketolides exhibit concentration-dependent killing, while higher concentrations do not enhance the killing by beta-lactams, macrolides and oxazolidinones [12,14–16]. Killing by these latter drugs is enhanced by longer exposure times, a pattern referred to as time-dependent killing.

Both in vitro and animal studies can also determine the impact of drug exposure on organism growth after drug exposure. However, only in vivo animal models are able to determine the time course of activity at the site of infection and the potential impact of host immune factors on antimicrobial activity. Both models have been utilized extensively for most antibacterial classes and many antifungals. Often the duration of these postantibiotic effects is of longer duration than when measured with in vitro techniques [16]. The longer PAEs may be due to sub-MIC effects, effect of serum factors, or slower growth in vivo than in the high nutrient environment of broth. Persistent in vivo effects have been observed for all antibacterials against staphylococcal species. Moderate to prolonged PAEs have been observed with inhibitors of protein and nucleic acid synthesis against Gram-negative pathogens and streptococci. On the other hand, minimal or no PAEs have been found with beta-lactam antibiotics against Gram-negative organisms and streptococci. The in vivo PAE of 11 h for azithromycin with a strain of *Streptococcus pneumoniae* is shown in Fig. 3 [14].

The time course activity characteristics of concentration effect, and postantibiotic effects, determine a pattern of antimicrobial activity of an antibiotic drug [13]. Three patterns of activity have been observed. The first pattern of activity is characterized by concentration-dependent killing and prolonged persistent effects. Higher drug concentrations result in more rapid and extensive organism killing. Dosing of drugs exhibiting

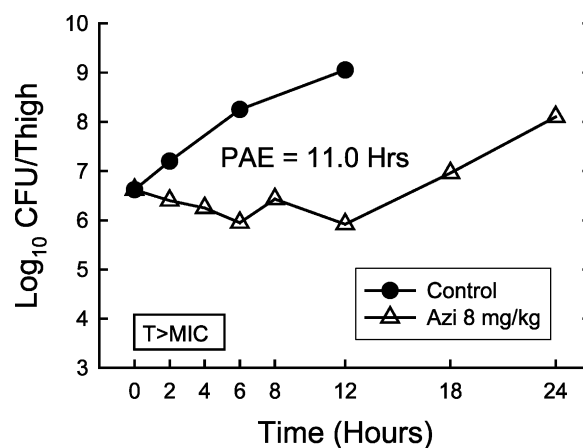


Fig. 3. In vivo postantibiotic effect with azithromycin at 8 mg/kg against *S. pneumoniae* ATCC 10813 in the thighs of neutropenic mice.

this pattern of activity is optimized by administration of large doses. Furthermore, dosing intervals can be lengthened because of prolonged PAEs. This pattern is predictive of activity of aminoglycosides, fluoroquinolones, ketolides, daptomycin, and the antifungal polyenes. The second pattern is characterized by time-dependent killing and minimal to moderate persistent effects. Higher concentrations of drugs demonstrating this pattern of activity do not enhance organism killing. Extending the duration of exposure optimizes antimicrobial activity with these drugs. Thus, the time that serum levels remain above the MIC is the PK/PD index correlating with treatment efficacy. A variety of drug classes including the beta-lactams and macrolides exhibit this pattern of activity. Time dependent killing and prolonged persistent effects characterize the final pattern of activity. Although higher drug concentrations do not enhance organism killing, the higher concentrations produce prolonged suppression of organism re-growth.

Impact of a Single Flucytosine Dose Interval on the Inter-relationship Among Pharmacokinetic/Pharmacodynamic Indices

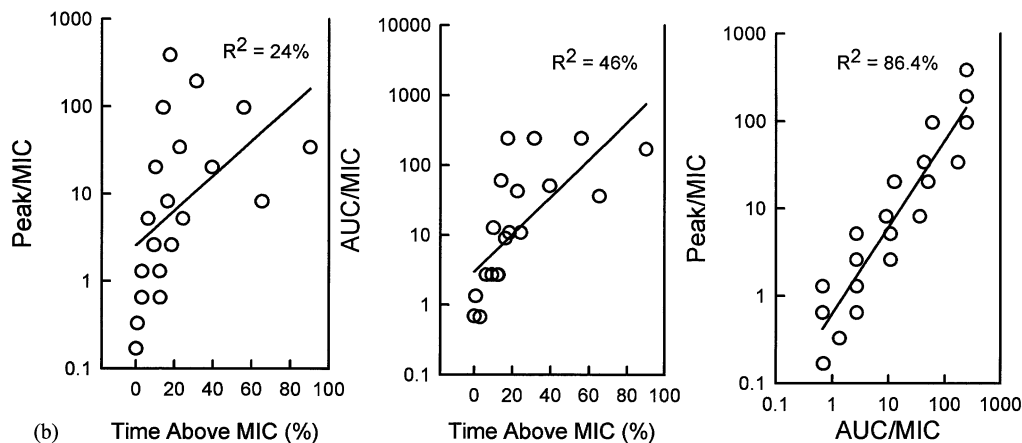
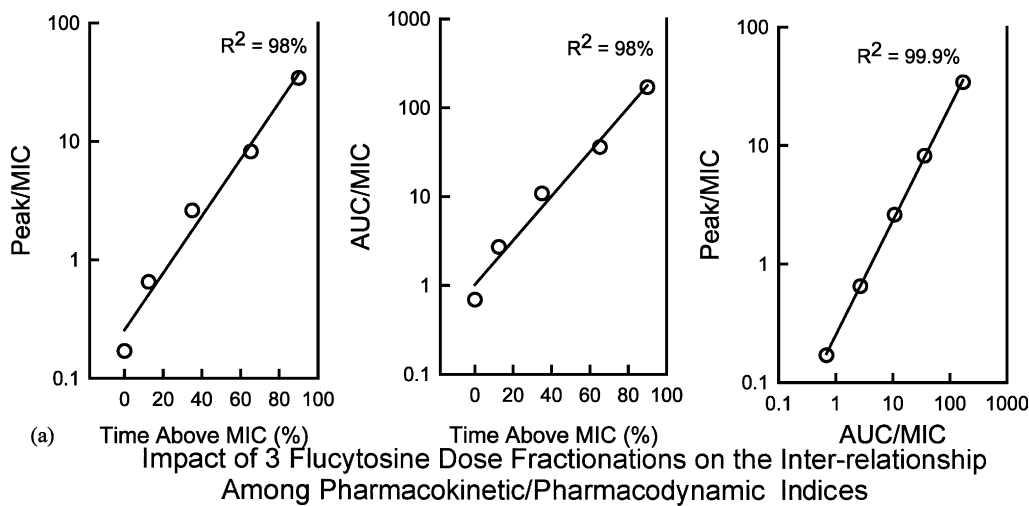


Fig. 4. (a) Impact of increasing dose using a single dosing interval on the inter-relationship among peak/MIC, AUC/MIC, and time above MIC for flucytosine. (b) Impact of increasing dose using a three different dosing intervals on the inter-relationship among peak/MIC, AUC/MIC, and time above MIC for flucytosine.

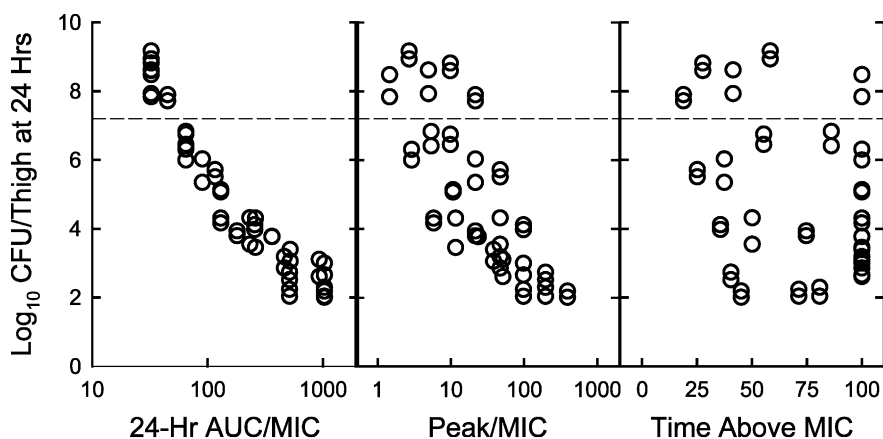


Fig. 5. Relationship among PK/PD indices for levofloxacin and \log_{10} CFU per thigh of *S. pneumoniae* after 24 h of therapy.

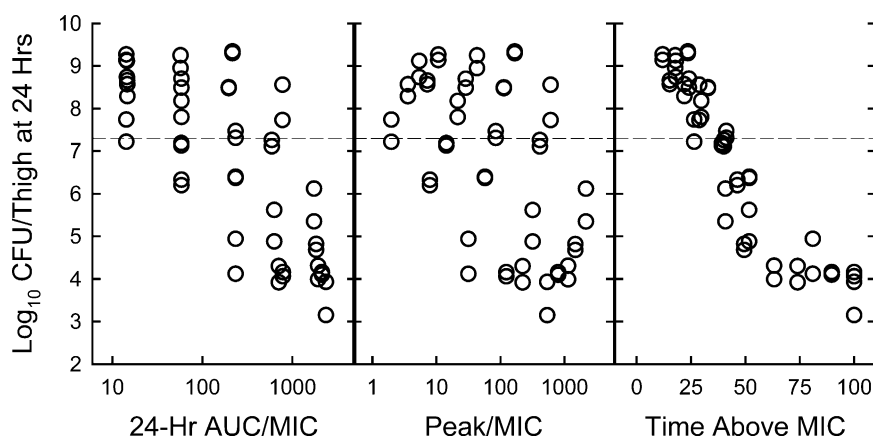


Fig. 6. Relationship among PK/PD indices for ceftazidime and \log_{10} CFU per lung of *K. pneumoniae* after 24 h of therapy.

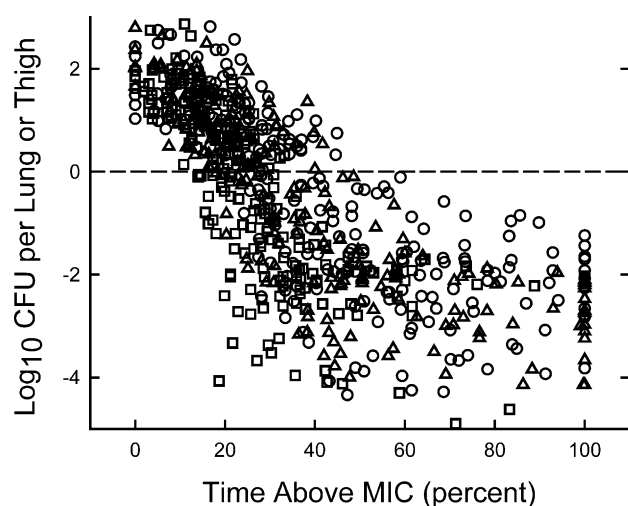


Fig. 7. Relationship between the change in \log_{10} CFU per thigh or lung for various pathogens following 24 h of therapy with different doses of penicillins (Δ), cephalosporins (\circ), and carbapenems (\square).

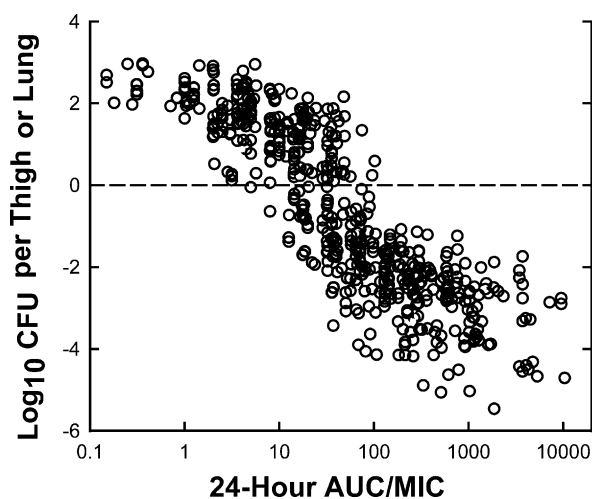


Fig. 8. Relationship between the change in \log_{10} CFU per thigh or lung for various pathogens following 24 h of therapy with different doses of various fluoroquinolones.

The goal of dosing with these drugs is to optimize the amount of drug. The PK/PD index, reflective of total drug amount or the area under the serum concentration curve (AUC) in relation to the MIC, is the index most closely associated with efficacy. This is the pattern observed for azithromycin, the tetracyclines, clindamycin, the glycylcyclines and the antifungal fluconazole.

1.3. PK/PD indices correlating with efficacy

Animal model studies have a distinct advantage over both in vitro models and clinical trials in the ability to discern which PK/PD dosing index is most closely associated with efficacy. Traditionally, the choice of an initial antimicrobial dose for a clinical trial is based upon achieving serum concentrations above the MIC of target organisms. Dosing intervals are often chosen roughly based on the serum elimination half-life. Subsequent clinical dosing studies may determine the efficacy of higher or lower drug doses, however, rarely would another dosing interval be examined. Analyses of treatment outcome in relation to the three PK/PD indices for these trials are rarely able to discern which is more predictive of outcome [13]. The inability of these multiple dose level, single dosing interval studies to make this distinction is due to the strong inter-relationship among the PK/PD indices. With each increase in dose level, the peak serum level, AUC, and time that serum levels remain above the MIC will all rise as demonstrated in Fig. 4a for flucytosine. By varying both dose and interval one may reduce the inter-relationship among the indices enough (see Fig. 4b) to discern which is more closely related to treatment outcome. The relationship between peak/MIC and AUC/MIC is the hardest to vary even with different dosing intervals.

Studies using four to six different dosing intervals have identified specific PK/PD indices that correlate with the in vivo activity of antibacterials and antifungals. As shown in Fig. 5, the activity of the fluoroquinolone, levofloxacin, against *S. pneumoniae* in the thighs

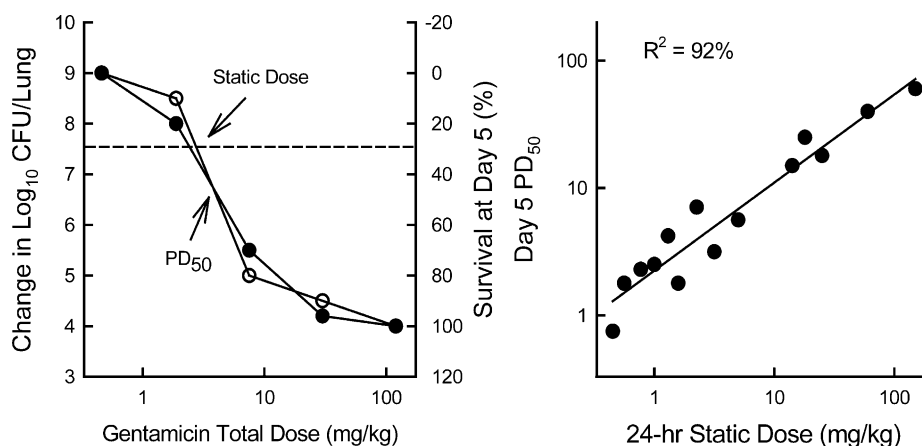


Fig. 9. Relationships between change in CFU per lung at 24 h and survival at day 5 with twice daily therapy with gentamicin (left panel) and between the 24 h static dose and the dose protecting 50% of mice from death (PD₅₀) for various beta-lactams, fluoroquinolones and aminoglycosides against *E. coli*, *K. pneumoniae* and *P. aeruginosa* (right panel).

of neutropenic mice correlated best with the 24 h AUC/MIC [17]. On the other hand, as shown in Fig. 6, the in vivo activity of ceftazidime against *Klebsiella pneumoniae* in the lungs of neutropenic mice correlated best with the duration of time serum concentration exceeded the MIC ($T > \text{MIC}$) [17].

1.4. Magnitudes of the PK/PD index required for efficacy

Animal models are also useful for definition of the dose level or PK/PD magnitude necessary for treatment efficacy. Clinical trials are most often not able to define the optimal dose level. Animal studies are able ethically to define the entire dose-response relationship and thus better define the PK/PD index magnitude predictive of treatment outcome. Furthermore, a variety of studies have suggested that the magnitude of the PK/PD index associated with efficacy is similar among various animal species including humans [13]. This demonstrates the ability of pharmacodynamics to correct for kinetic variability among species. This should not be surprising,

as the target of an antimicrobial is in the pathogen and not in the animal species. The definition of effective dosing index magnitudes in various animal models have been especially useful for design of optimal dosing regimens in clinical scenarios that are encountered infrequently in treatment trials [12,13]. For example, despite the increasing incidence drug resistant pathogens, most clinical trials are unable to enrol enough patients with these resistant pathogens to feel comfortable predicting treatment efficacy. However, animal model studies are able to define the relationship between index magnitude and effect against organisms with widely varying MICs [12]. In addition, these studies can define efficacy against organisms with reduced susceptibility due to specific resistance mechanisms.

Animal model studies have also been useful for determining the impact of a number of treatment variables on PK/PD index magnitude targets. These variables include, pathogen species, drug-resistant pathogens, site of infection, drugs with similar mechanisms of activity, the treatment endpoint, and various

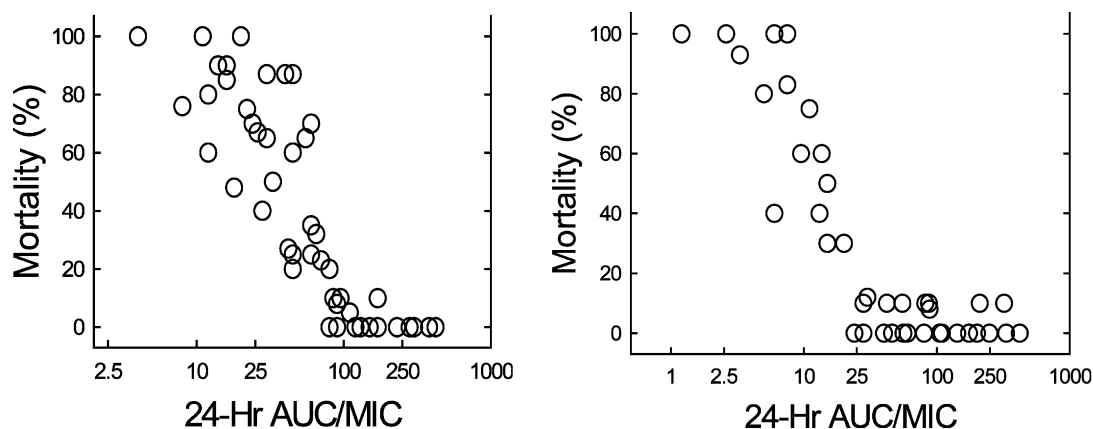


Fig. 10. Relationships between mortality at the end of therapy and the 24 h AUC/MIC of fluoroquinolones with multiple pathogens (left panel) in different animal models (mostly immunocompromised) and with *S. pneumoniae* in non-neutropenic models (right panel).

Relationship Between Quinolone AUC/MIC and Mortality at 7–12 Days After the End of Therapy

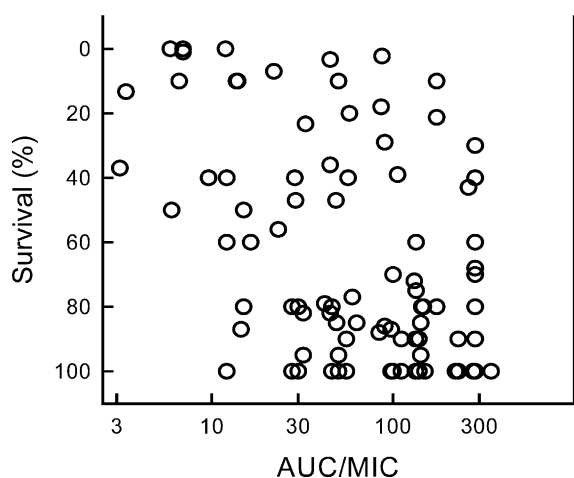


Fig. 11. Relationship between mortality at 7–12 days after the end of fluoroquinolone therapy and the 24 h AUC/MIC.

host immune defects. Multitudes of studies have demonstrated that the dosing index magnitude predictive of outcomes is similar for drugs within the same drug class and for most pathogens [13]. This has been most convincingly demonstrated for numerous beta-lactam and fluoroquinolone antibiotics [2,13,14]. Fig. 7 illustrates the results of multiple dosing studies with penicillins, cephalosporins and carbapenems against staphylococci, pneumococci and Gram-negative bacilli in the lungs and thighs of neutropenic mice [18]. While there is significant overlap of the results for the different drugs, there is a trend for carbapenems requiring less $T > MIC$ than penicillins, which in turn require slightly less $T > MIC$ than cephalosporins. Most of the drugs require $T > MIC$ from 25 to 50% of the dosing interval for 1–2 log kills. Fig. 8 illustrates the results of multiple dosing studies for fluoroquinolones with similar pathogens in the lungs and thighs of neutropenic mice [19]. For these drugs, a bacteriostatic effect was observed when the 24-h AUC/MIC varied from 10 to 80 with a mean of around 30. The kinetic parameter target magnitude associated with efficacy has also been shown to be similar in thigh and lung infection models as shown in Figs. 7 and 8 above.

The actual endpoint used for measuring antimicrobial activity can also influence interpretation of results. Many studies in the past have used survival as the major endpoint for treatment outcome. However, organism counts, especially those performed after 24 h, will often provide a wide range of responses that allows for use of lower numbers of animals to differentiate between treatment outcomes. Several studies have demonstrated a strong relationship between bacterial

numbers and survival. As shown in the left panel of Fig. 9, the change in \log_{10} CFU per lung after only 24 h of twice daily therapy with gentamicin against *K. pneumoniae* was very similar to the survival data obtained after 5 days of twice-daily therapy [20,21]. The right panel of Fig. 9 shows that there is an excellent correlation between the static dose derived from bacterial numbers at 24 h and the daily dose resulting in 50% survival on day 5. The symbols represent data with various beta-lactams, fluoroquinolones and aminoglycosides against *Escherichia coli*, *K. pneumoniae* and *P. aeruginosa*.

A review of survival studies for fluoroquinolones from the literature including our own demonstrate a good correlation between mortality and the 24-h AUC/MIC [2,19]. Only those studies that treated animals for at least 48 h reported survival results at the end of therapy, had greater than 80% mortality in untreated controls and provided pharmacokinetic data were included. This included studies of pneumonia, peritonitis, sepsis and soft-tissue infection in immunocompromised mice, rats and guinea pigs, and using various strains of Gram-negative bacilli and pneumococci. As shown in the left panel of Fig. 10, 24-h AUC/MIC values of 30 were associated with about 50% survival, while maximal survival was observed with values of 100 or greater. Similar studies performed with non-neutropenic animals against *S. pneumoniae* are shown in the right panel of Fig. 10. In this case, maximum survival was observed at values of 25 or greater [19].

One treatment variable that can impact the amount of antibiotic necessary for efficacy is the host immune state. For example, several studies have suggested that the PK/PD index magnitude necessary for successful therapy is reduced in animal models by the presence of neutrophils [2,11]. The lower 24-h AUC/MIC for fluoroquinolones required for survival of non-neutropenic animals infected with pneumococci in Fig. 10 above is likely due to the effect of neutrophils. This has been demonstrated with other antimicrobials as well. The magnitude of the static dose for macrolides, azalides and streptogramins against *S. pneumoniae* was reduced 1.6- to 3.8-fold in non-neutropenic compared with neutropenic mice [11].

Many studies that use survival as an outcome follow mortality for prolonged periods of time after therapy has ended. This practice may allow organisms that have not been eradicated to regrow and produce mortality. This is especially a problem with prolonged neutropenia where organisms would not even have neutrophils to reduce their regrowth. Fig. 11 illustrates the relationship between survival at 7–12 days after the end of fluoroquinolone therapy and the 24-h AUC/MIC [2]. The majority of these studies were performed in neutropenic animals.

2. Conclusions

Animal models have been very useful for determining the (1) relationship between serum and tissue concentrations, (2) time-course of antimicrobial activity in vivo, (3) PK/PD indices correlating with efficacy and (4) magnitudes of the PK/PD index required for efficacy. Although these animal studies have demonstrated that the PK/PD indices can vary for different classes of antimicrobials, the magnitude of the target required for bacteriological cure and survival is relatively similar for various sites of infection, various pathogens, and various drugs within the same class, provided free drug levels are used. Despite the variety of techniques and models, there is marked consistency in the PK/PD data in animals.

References

- [1] Redington J, Ebert SC, Craig WA. Role of antimicrobial pharmacokinetics and pharmacodynamics in surgical prophylaxis. *Rev Infect Dis* 1991;13(Suppl. 10):790–9.
- [2] Craig WA, Dalhoff A. Pharmacodynamics of fluoroquinolones in experimental animals. In: Kuhlmann J, Dalhoff A, Zeiler HJ, editors. *Handbook of experimental pharmacology: quinolone antibacterials*, vol. 127. Heidelberg: Springer, 1998:207–32.
- [3] Craig WA, Suh B. Protein binding and the antimicrobial effects: methods for the determination of protein binding. In: Lorian V, editor. *Antibiotics in laboratory medicine*. Baltimore: Williams and Wilkins, 1991:367–402.
- [4] Merrikin DJ, Briant J, Rolinson GN. Effect of protein binding on antibiotic activity in vivo. *J Antimicrob Chemother* 1983;11:233–8.
- [5] Craig WA, Redington J, Ebert SC. Pharmacodynamics of amikacin in vitro and in mouse thigh and lung infections. *J Antimicrob Chemother* 1991;27(Suppl. C):29–40.
- [6] Ebert SC, Leggett J, Vogelmann B, Craig WA. Evidence for a slow elimination phase for penicillin G. *J Infect Dis* 1988;158:200–3.
- [7] Leggett JE, Ebert S, Fantin B, Craig WA. 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* 1991;(Suppl 74):179–84.
- [8] Leggett JE, Fantin B, Ebert S, et al. Comparative antibiotic dose-effect relations at several dosing intervals in murine pneumonitis and thigh-infection models. *J Infect Dis* 1989;159:281–92.
- [9] Kapusnik JE, Hackbarth CJ, Chambers HF, Carpenter T, Sande MA. Single, large daily dosing vs. intermittent dosing of tobramycin for treating experimental pseudomonas pneumonia. *J Infect Dis* 1988;158:7–12.
- [10] Vogelmann B, Gudmundsson S, Leggett J, et al. Correlation of antimicrobial pharmacokinetic indices with therapeutic efficacy in an animal model. *J Infect Dis* 1988;158:831–47.
- [11] Pechere M, Letarte R, Pechere JC. Efficacy of different dosing schedules of tobramycin for treating a murine *Klebsiella pneumoniae* bronchopneumonia. *J Antimicrob Chemother* 1987;19:487–91.
- [12] Andes D, Craig WA. In vivo activities of amoxicillin and amoxicillin-clavulanate against *Streptococcus pneumoniae*: application to breakpoint determinations. *Antimicrob Agents Chemother* 1998;42:2375–9.
- [13] Craig WA. Pharmacokinetic/pharmacodynamic indices: rationale for antibacterial dosing of mice and men. *Clin Infect Dis* 1998;26:1–12.
- [14] Craig WA. Postantibiotic effects and the dosing of macrolides, azalides, and streptogramins. In: Zinner SH, Young LS, Acar JF, Neu HC, editors. *Expanding indications for the new macrolides, azalides and streptogramins*. New York, USA: Marcel Dekker, 1997:27–38.
- [15] Craig WA. Dose the dose matter. *Clin Infect Dis* 2001;22(Suppl. 3):233–7.
- [16] Craig WA, Gudmundsson S. Postantibiotic effect. In: Lorian V, editor. *Antibiotics in laboratory medicine*. Baltimore: Williams and Wilkins, 1996:296–329.
- [17] Craig WA. Pharmacodynamics of antimicrobials. In: Nightengale C, Ambrose P, editors. *Pharmacodynamics of Antimicrobials*.
- [18] Craig WA, Watanabe Y. In vivo pharmacodynamic activity of temafloxacin. Abstr 39, 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC.
- [19] Craig WA. In vivo pharmacodynamics of the fluoroquinolones. *Clin Infect Dis*, in press.
- [20] Leggett JE, Ebert S, Fantin B, Craig WA. A sigmoid dose-response model using bacterial counts predicts dose-survival for *Klebsiella pneumoniae* in neutropenic mice. Abstr 313, 29th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC.
- [21] Vesga O, Andes D, Craig WA. Correlation of bacterial counts at 24 h with survival after 45 days of therapy. Second International Symposium on Infection Models in Antimicrobial Chemotherapy, Reykjavik, Iceland, 1996.