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PK/PD models in antibacterial development

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Abstract

There is an urgent need for novel antibiotics to treat life-threatening infections caused by bacterial 'superbugs'. Validated *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) and animal infection models have been employed to identify the most predictive PK/PD indices and serve as key tools in the antibiotic development process. The results obtained can be utilized for optimizing study designs in order to minimize the cost and duration of clinical trials. This review outlines the key *in vitro* PK/PD and animal infection models which have been extensively used in antibiotic discovery and development. These models have shown great potential in accelerating drug development programs and will continue to make significant contributions to antibiotic development.

Keywords

PK/PD; antibacterial; drug discovery

Introduction

Rapidly increasing antibiotic resistance and the lack of new antibiotics in the drug discovery pipeline are presenting a significant unmet global medical need [1]. Antimicrobial resistance has been identified as one of the three greatest threats to human health. An urgent global call for the discovery of new antibiotics, *The 10 × '20 Initiative*, has been made recently [1]. In antibiotic discovery and development, pharmacokinetic/pharmacodynamic (PK/PD) and animal infection models play essential roles and bridge the gap between *in vitro* susceptibility and clinical evaluations of new antibiotics. Identification of PK/PD relationships in an early discovery stage provides a quantitative tool to enable rational go or no-go decision making and predictions of clinical pharmacological profiles of superior

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leads. This review outlines the key PK/PD models that have been extensively used in antibiotic discovery and development.

In vitro PK/PD models

In vitro PK/PD models essentially fall into one of two categories: one-compartment or two-compartment models (Figure 1) [2,3]. One-compartment models typically consist of a central reservoir containing the organism, a diluent reservoir and a waste reservoir. Drug is administered to the central reservoir with drug elimination achieved by pumping drug-free media into the central reservoir; this setup, while necessary for mimicking the PK of antibiotics in patients (i.e. simulation of the desired antibiotic half-life), simultaneously eliminates bacteria. This unintended consequence can be problematic for antibiotics with short elimination half-lives and is the primary disadvantage of one-compartment PK/PD models. To overcome this problem filters have been utilized to prevent bacterial loss, but are prone to blockage [4].

Two-compartment PK/PD models are similar to one-compartment models, but prevent bacterial elimination by physically separating bacteria from the central reservoir within a small peripheral compartment (typically 10 – 20 mL). The most common example is the hollow fiber infection model (HFIM) containing thousands of small tubular fibers (filters) in a cartridge through which medium is pumped [5]. Pores on the fibers retain the microorganisms while allowing the free diffusion of drugs and other molecules (e.g. glucose). Drug is administered into, and eliminated from, the central reservoir with antibiotic concentrations equilibrating rapidly with the peripheral (bacterial containing) compartment. Importantly, both absorption and elimination kinetics of the antibiotic under investigation can be precisely and independently controlled. The versatility of both one- and two-compartment models allows for the simulation of virtually any desired elimination half-life observed in patients.

These PK/PD models have played an important role in the determination of the key PK/PD indices driving antibacterial activity (i.e. C_{max}/MIC [the peak concentration divided by the MIC], AUC/MIC [the area under the concentration-time curve over 24 h in steady-state divided by the MIC] or $T_{>MIC}$ [the cumulative percentage of a 24-h period that the drug concentration exceeds the MIC at steady-state pharmacokinetic conditions]) [2]. Identification of the most predictive PK/PD index and the associated values required for different magnitudes of killing is essential for the rational design of optimal dosing strategies in animal and clinical studies. Dose-fractionation studies in *in vitro* PK/PD models are more easily performed than in animal models. A recent example is the work by Bergen *et al.* that identified AUC/MIC as the main driver of antibacterial activity for colistin [6]. This information subsequently contributed to the first scientifically-based dosing guidelines for colistin in critically-ill patients [7]. Such *in vitro* dose-fractionation is increasingly applied to dosage regimen optimization of other antibiotics [8]. The PK/PD information obtained is crucial for designing optimal dosing strategies for further evaluations in animal models and clinical trials.

In vitro PK/PD models are also increasingly being used in the assessment of the emergence of resistance with mono- and combination antibiotic therapy [9–12], and demonstrate that emergence of resistance is a complex interplay of the PK and PD of antibiotics [13,14]. Thus the PK profiles simulated in PK/PD models provide more clinically relevant information than static models. The utility of PK/PD models in this regard is exemplified in the study by Tam *et al* [13]. Using a hollow fiber infection model, it was demonstrated that, in a heterogeneous bacterial population with multiple subpopulations of varying drug susceptibility, low to medium exposures (based on AUC/MIC) of quinolones selectively

amplified resistant subpopulation(s) whereas high drug exposures suppressed this. Bergen *et al*. demonstrated that of three dosage regimens each providing a similar exposure to colistin, emergence of resistance was substantially greater and occurred earlier with the two colistin regimens employing the longer dosage intervals [15]. Additionally, *in vitro* PK/PD models have been employed to identify antibiotic breakpoints deemed crucial for suppression of resistance development [16].

In addition to being less cost- and resource-intensive, *in vitro* PK/PD models permit investigations of considerable duration (e.g. weeks) that may not be feasible in animals. Furthermore, PK/PD models allow for the use of high inocula without the ethical concerns associated with excessive early mortality of the animals; the latter is particularly important for the investigation of resistance development as a high bacterial load (e.g. 10^8 CFU/mL) is usually required to increase the probability of detection of resistant mutants [14]. In addition, these models can be used to examine microorganisms for which animal models are not well established. Results obtained from *in vitro* PK/PD models have shown good correlations with human and animal data [17,18]. The lack of immune components in *in vitro* models is both a limitation and an advantage. While this presents difficulties in extrapolating results to immunocompetent hosts, *in vitro* models permit the direct evaluation of the activity of antibiotics themselves in the absence of host defenses, mimicking the situation in the immunocompromised. It is for this reason that PK/PD models have been particularly useful in the study of anti-tubercular drugs [18].

In summary, *in vitro* PK/PD studies provide important insight into the therapeutic potential of lead compounds in early antibiotic development, and assist in the design of optimal dosage strategies for animal studies and clinical trials.

In vitro biofilm models

Microorganisms are frequently biofilm-embedded in nature and also in the clinic such as in catheter or prosthetic joint infections, chronic sinusitis and infective endocarditis [19]. Biofilm can result in increased antimicrobial tolerance by altering bacterial metabolism, retarding the diffusion of antibiotics, increasing the enzymatic-inactivation of antibiotics in the extracellular matrix, and impairing bacterial clearance by the immune system [19]. In *in vitro* biofilm models, factors including restriction of nutrients and oxygen, surface material, shearing force and the age of the biofilm may significantly influence the maturity of the biofilm and its response to antimicrobials [20–22]. The classic concepts of MIC and minimal bactericidal concentration for planktonic cells have a poor clinical correlation in a biofilm scenario. Minimal biofilm inhibitory (MBIC) and eradicative concentrations (MBEC) more accurately reflect the activity of antimicrobials in biofilm [23]. Measurements of MBIC and MBEC can be achieved by microtiter plate-based models using automatized technology. The Calgary device [23] has been widely used, and numerous variations (e.g. addition of magnetic beads to the media used in the Biofilm Ring Test [24] or microcalorimetric assays [25]) have been recently incorporated into this static biofilm model. However, for examining the anti-biofilm PK/PD of antibacterials, dynamic models are required to mimic antibacterial PK *in vivo*. In the plug flow reactors, microbiological broth flows in one direction and solutes diffuse in a radial direction [21]. Another recent development is the drip flow biofilm reactor that is able to grow biofilm under low shearing forces [21]. Similarly, microfluidic devices (e.g. BioFlux) allow multiple parallel experiments for growing biofilm under low flow rates and shearing forces [22]. In continuous flow stirred tank reactors, homogenous mixing and diffusion of solutes occurs throughout the reactor [20]. Two representative examples are the Rotating Disk Reactor [26] and the CDC Biofilm Reactor [27]. In addition, in these models imaging techniques (e.g. advanced fluorescence microscopy and integrated nuclear magnetic resonance and confocal

laser scanning microscopy) are commonly used for evaluating antimicrobial diffusion, and changes in the biofilm ultra-structure and on viable but non-culturable bacteria after antibiotic treatment [28–30].

Animal infection models

Animal infection models serve an important role in simulating the pathophysiology of infections in patients and as a platform for preclinical assessments of new antibiotics, as well as optimizing antibiotic use [31]. Pertaining to this review, animal models have been instrumental for evaluating antimicrobial PK/PD, notably the relationships between *in vitro* activity, bacterial growth, size of the inoculum, the timing of treatment, PK and *in vivo* efficacy [32]. Disadvantages of animal models include the variations in the PK of antibiotics compared to that in humans. In attempts to simulate human PK and usually prolong the half-life of the drug in animals, multiple doses or inducing transient renal impairment in animals by administration of uranyl nitrite can be employed [33]. In addition, allometric scaling should be considered when designing dosage regimens in animals. This section provides a practical overview of the most commonly used animal models in antibiotic drug discovery.

Thigh infection models

The mouse thigh infection model is the most common animal model to examine antibiotic PK/PD relationships [33,34]. The model is relatively inexpensive and reproducible. Mice are rendered neutropenic by treatment with cyclophosphamide on days -4 and -1, producing neutropenia by day 0 [33,34]. Log-phase bacterial cells (normally 10^5–10^6 colony forming units [CFU], depending on bacterial strains) are injected into each thigh under light anesthesia. An important consideration is the time difference between inoculation and the commencement of therapy. The tested compound is administered over 24 h with multiple dosing regimens depending on the half-life and the PK/PD indices under investigation. The efficacy of the antibacterial agent is commonly determined by subtracting the $\log_{10}$ CFU/thigh at 24 h of the treated mice from that of the control mice at 0 h. PK/PD indices of $T_{>\mathrm{MIC}}$, AUC/MIC or C_{max}/MIC can be related to the *in vivo* efficacy, most commonly by a sigmoid model [34]. Notable examples of the application of the mouse thigh infection model to study PK/PD relationships for antibiotic development include cephalosporin PPI-0903 [35] and linezolid [36]. In the linezolid study, it was revealed that a dosage regimen of 600 mg twice daily (AUC/MIC of 50 to 100) would be effective against pathogens with MICs as high as 2 to 4 mg/L [36].

Septicemia models

This model has been instrumental for *in vivo* efficacy of numerous antibiotics [37,38]. The model has been implemented across a number of animal species; however, for reasons of economy mice and rats are most commonly used. The simplicity of the endpoint analysis lends the mouse septicemia model to the routine use for preclinical *in vivo* efficacy assessment of novel antimicrobials [38]. For mice, in most instances, the model involves rendering the animal neutropenic through the administrations of 100–150 mg/kg of cyclophosphamide once a day for 3 days. The unanesthetized animal is then infected by an intraperitoneal injection of 0.1–0.5 mL of a log-phase bacterial suspension. Antibiotic(s) is administered by subcutaneous injection 1 h postinoculation over multiple dosage regimens for a period of up to 72 h. Other drug administration routes can be used depending on the prospective formulation of the compound. Endpoints for this model can be morbidity (% survival) and bacterial load (CFU) in the blood. Compared to the thigh infection model, the mouse septicemia model is significantly less time consuming and labor intensive as tissue homogenization and filtration are not required for viable counting.

Endocarditis models

Bacterial endocarditis can be a very difficult infection to treat due to inaccessibility of organisms within the core of the vegetations to the immune system and poor penetration of antibiotics into the infected endocardial vegetations [39]. The latter can also set ideal conditions for bacteria to develop resistance. Moreover, bacteria within the core of the vegetations display low metabolic activity rendering them less susceptible to antibiotics [40]. Endocarditis animal models have been developed in several species including mouse, rat, rabbit, pig, dog and opossum [41]. Endpoints used in this model include CFU/vegetation and morbidity; blood samples are also collected to test for sepsis and relapse of infection following treatment. Endocarditis models have been extensively used for antibacterial PK/PD studies [41]. For fluoroquinolones, it was reported that an AUC/MIC ≥100 is required for bacterial clearance over 3–6 days of therapy [41].

Urinary tract infection (UTI) models

UTI is a significant urologic disease in women, predominantly caused by uropathogenic *Escherichia coli* from the intestinal flora that colonize the urethra and bladder [42]. UTI may even ascend from the bladder to the kidneys causing permanent damage and scarring [43]. Several animal models of ascending unobstructed UTI have been developed for antibacterial pharmacology and discovery [44]. Female mice are routinely used to simulate ascending UTI in women; however, male mice can also be employed [45]. After the animal is anesthetized, a catheter is inserted into the urethra and a needle is inserted into the catheter opening through which ~50 μL of bacterial suspension is delivered (usually 10^7–10^9 CFU/mouse). The mouse should not be given liquids for 1 h prior to and after bacterial challenge to reduce urine output. Careful attention should be paid to the growth media used for preparation of the inoculum as certain medium conditions provide for the expression of virulence factors required for uropathogenesis. The infection usually peaks one day post challenge and resolves over 2–3 weeks, depending upon the bacterial strain, the genetic background of inbred mice, and the absence of inoculation-associated vesicoureteral reflux. The endpoints are usually bacterial cultures of bladder and kidney homogenates. Additional parameters monitored may include morbidity and blood cultures, while homogenates of liver and spleen can also be taken to monitor dissemination of the infection outside the urinary tract. The mouse UTI model was recently employed to demonstrate the *in vivo* efficacy of ACHN-490, a new aminoglycoside with good *in vitro* activity against MDR Gram-negative and select Gram-positive pathogens [38]. ACHN-490 treatment (0.125–8 mg/kg/12 h for 3 days) effectively reduced $\log_{10}$CFU counts in the kidneys, bladder and urine of treated animals [38].

Wound infection models

Infection remains the major cause of morbidity in wound patients worldwide [46]. Numerous external traumatic wound infection models have been developed to simulate various forms of traumatic injury and evaluate antibacterial treatment [46]. Examples of animal wound infection models include skin abrasions, burns and excision wounds. Albino Hartley guinea pigs are typically used for wound models; their dorsal hair is clipped and a black grid is drawn on the back of the animal where the lesions are created. The main factors which determine the severity of the traumatic wound infection model include bacterial inoculum, size of the wound and immune-competence of the animals. The end-point for these models usually includes histopathological examination of sections of lesions and counting of viable bacteria recovered from the inoculation sites to determine the inoculation producing 50% probability of infection (ID_{50}). ID_{50} values are determined by logistic regression from a plot of the infection rate *versus* the bacterial inoculum size, and can be employed to access the efficacy of antibacterial agents. The assessment of antibacterial

agents in wound models has generally yielded good correlation between their *in vitro* activity and *in vivo* efficacy in humans [47].

Animal biofilm models

Several biofilm-related animal models have been developed with or without the addition of foreign material, including central venous catheter models, subcutaneous foreign body infection models and osteomyelitis infection models [20]. The infection may be established by direct inoculation into a specific organ or space (e.g. the otitis media model), manipulation of the infection site (e.g. cortical bone drilling before inoculation in osteomyelitis models), or implantation of a foreign body (e.g. device-related osteomyelitis) [20]. The microorganisms inoculated are usually planktonic but capable of attaching to surfaces and developing biofilm. Sessile biofilm-embedded microorganisms have also been used for inoculation to mimic specific clinical scenarios [48]. Recently, an *in vivo* polymicrobial biofilm wound infection model was developed to study interspecies interactions in biofilm and their relation to wound chronicity [49].

In addition, a number of recent animal infection models have been adapted for the real-time monitoring of infections using luminescent bacteria [50,51]. This allows for the monitoring of infections in live animals in a non-invasive manner. Nevertheless, the sensitivity of bioluminescence is generally lower compared to viable counting methods; hence, such models may not be able to differentiate a marked bactericidal action from mild antibacterial effect.

Antimicrobial PK/PD modeling

State-of-the-art data analysis to optimize the data gained from the *in vitro* and animal models is critical for antibiotic development. Traditional PK/PD target approaches aim to maximize $T_{>\mathrm{MIC}}$, AUC/MIC or C_{max}/MIC with the targets for stasis and different magnitudes of bacterial killing derived from pre-clinical models. Combined with population PK modeling, the PK/PD target approach allows the prediction of the likelihood of target attainment in a patient population (including for dosage regimens not previously studied in clinical trials) [52]. More recently, mechanism-based mathematical (MBM) models [53] have been developed to incorporate (a) multiple biologically relevant mechanisms (e.g. antibacterial action and resistance), (b) concentration-time courses of single or multiple antibiotics, (c) effects of antibiotic exposure on bacterial killing and emergence of resistance in heterogeneous bacterial sub-populations with different antibiotic susceptibilities, and (d) effects of the immune system. Based on *in vitro* PK/PD data (e.g. hollow fiber infection model), MBM models can establish a quantitative relationship between PK profiles in patients and the time course of bacterial killing and resistance for further pre-clinical and clinical evaluations.

Conclusion

One of the significant challenges in antibiotic development is to establish the correlation between *in vitro* susceptibility and clinical efficacy. Hence, validated *in vitro* PK/PD and animal infection models serve as key tools in the antibiotic development process and have been widely employed for identifying the most predictive PK/PD indices. After analysis using comprehensive mathematical modeling, the results obtained set a quantitative basis for optimizing study designs in order to minimize the cost and duration of expensive clinical trials. In summary, *in vitro* PK/PD and animal infection models have shown great potential in increasing success rates and accelerating the drug development process, and will continue to make a significant contribution to the search for new antibiotics.

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Abbreviations

MIC	minimum inhibitory concentration
AUC	the area under the concentration-time curve
C_{max}	peak concentration
PK	pharmacokinetics
PD	pharmacodynamics
AUC/MIC	the area under the concentration-time curve over 24 h in steady-state divided by the MIC
C_{max}**/MIC**	the peak concentration divided by the MIC
$T_{>MIC}$	the cumulative percentage of a 24-h period that the drug concentration exceeds the MIC at steady-state pharmacokinetic conditions

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Highlights

- *In vitro* PK/PD models are less cost- and resource-intensive and permit investigations of considerable duration not feasible in animals.
- Mouse thigh infection model is the gold standard for antibacterial PK/PD.
- Animal infection models play a critical role in the preclinical assessment of novel antibiotics.

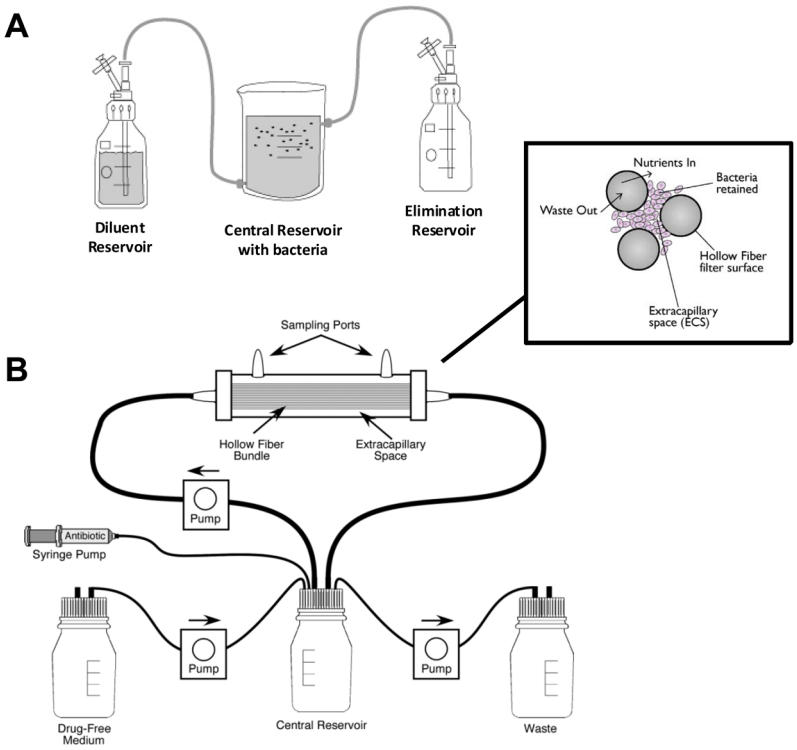

Figure 1.

In vitro PK/PD models. (A) The one-compartment model. The volume remains constant but the test organism is not constrained. (B) Hollow fiber two-compartment model. Bacterial cells reside in the hollow fiber cartridge. The nutrient broth continually re-circulates through the central reservoir and cartridge. Drug is administered to the central reservoir and the elimination kinetics is controlled by the addition of fresh drug-free medium to the central reservoir. Figures adapted from reference [5] with permission.