How preclinical infection models help define antibiotic doses in the clinic

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Abstract

Appropriate dosing of antibiotics is key in the treatment of bacterial infections to ensure clinical efficacy while avoiding toxic drug concentrations and minimizing emergence of resistance. As collection of sufficient clinical evidence is difficult for specific patient populations, infection types and pathogens, market authorization, dosing strategies and recommendations often rely on data obtained from in vitro and animal experiments. The aim of this review is to provide an overview of commonly used preclinical infection models, including their strengths and limitations. In vitro, static and dynamic time-kill experiments are the most frequently used methods for assessing pharmacokinetic/pharmacodynamic (PK/PD) associations. Limitations of in vitro models include the inability to account for the effects of the immune system, and uncertainties in clinically relevant bacterial concentrations, growth conditions and the implications of emerging resistant bacterial populations during experiments. Animal experiments, most commonly murine lung and thigh infections models, are considered a necessary link between in vitro data and the clinical situation. However, there are differences in pathophysiology, immunity, and PK between species. Mathematical modeling in which preclinical data are integrated with human population PK can facilitate translation of preclinical data to the patient’s clinical situation. Moreover, PK/PD modeling and simulations can help in the design of clinical trials aiming to establish optimal dosing regimens to improve patient outcomes.

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1. Introduction

Correct dosing of antibiotics is imperative to ensure efficacy and avoid toxicity. Given the paucity of novel antibiotics, suppression of resistance is also an important consideration. Randomized trials targeting a general or more specific patient population, infection type and pathogen are ideal to support treatment decisions and dosing strategies. As these targets are diverse, with differing immunological status and other host factors, pathogen susceptibility, and severity and site of infection, such trials are difficult to perform [1]. Thus, for many clinical scenarios, data from in vitro and animal models are relied upon. Preclinical studies remain the basis for determining dosing principles for antibiotics and are critical to informing and optimizing the design of early clinical studies.

The development of a new antibiotic therapy begins in the laboratory. For the most promising compounds, in vitro dynamic time-kill data are employed to determine pharmacokinetic and pharmacodynamic (PK/PD) associations and potential PK/PD targets. Although efficient, the translation of in vitro results to the clinic is hampered for several reasons, most notably the lack of in vivo physiological processes. Evaluation using animal infection models thus remains an essential link between in vitro assessment and clinical studies. The integration of basic PK/PD associations, defined from in vitro and animal studies, with human PK studies by mathematical modeling and simulation that considers patient variability creates a powerful tool to inform dosing strategies and has become a cornerstone for setting clinical susceptibility breakpoints [2,3].

As antibiotic dosing largely relies on preclinical data, it is important to understand the strengths and limitations of available methods. Herein are described commonly used in vitro and animal infection models and new approaches that may facilitate translation of results to the clinic, thereby supporting optimal dosing.
regimens for existing antibiotics and accelerating the development of new drugs.

2. In vitro PK/PD models

Advantages of in vitro models include the opportunity to determine PK/PD associations between antibiotic exposure and antibacterial activity in a controlled environment. Static methods have a relatively low workload compared with dynamic in vitro and animal models. Dynamic infection models are closer to the clinic as antibiotic concentrations mimicking human antibiotic PK can be used.

2.1. Static experiments

Determination of minimum inhibitory concentrations (MICs) using standard methods, e.g., broth microdilution, is the first step in assessing antibiotic activity [4]. The methods applied for MIC determination normally use lower working volumes and bacterial inocula, and have a higher limit of detection of bacterial concentrations compared with other in vitro assays. The MIC value provides limited information on the dynamics of bacterial killing and growth during antibiotic exposure as it is normally based on a single readout after 16–20 h of incubation and there is high variability in results [5]. Monitoring of bacterial growth dynamics by repeated assessment of bacterial concentrations during exposure to static antibiotic concentrations is employed to describe antibiotic PD activity in terms of killing and growth of the targeted pathogen. The static time-kill method is probably the most frequently used assay for assessing antibiotic PK/PD [6]. By sampling for viable counts, bacterial reductions and regrowth can be determined at multiple time-points based on bacterial inoculations (colony forming units [CFU]/mL). This method also enables phenotypic and genotypic characterization of surviving bacterial populations and resistance development during experiments, e.g. by repeated MIC determination, population-analysis profiling using antibiotic-containing plates, and whole-genome sequencing.

Other techniques that require less manual work may be preferable when multiple antibiotics or drug concentrations are to be tested. For this purpose, several non-plating methods have been developed, including spectrophotometric assays where optical density (OD) is used as a marker for changes in bacterial density. Checkerboards are frequently used to efficiently evaluate synergy with antibiotic combinations at multiple concentrations, using an optical readout at 24 h; definitions of synergy and antagonism are defined based on the fractional inhibitory concentration index (FICI) [7]. Time-lapse microscopy, e.g., the oCelloScope system (BioSense Solutions ApS, Farum, Denmark) in which bacterial density is automatically monitored using image analysis, can be used to screen for combination effects against multidrug-resistant Gram-negative bacteria [8–9]. This method enables assessment of bacterial growth dynamics and morphological changes during experiments and has a lower limit of detection of bacterial growth than checkerboards. Still, time-kill experiments provide more detailed information on bacterial killing and emergence of resistance [10].

2.2. Dynamic experiments

In dynamic time-kill experiments, antibiotic concentrations are tailored to mimic typical human PK profiles of the study drug in the targeted patient group, thereby facilitating translation of in vitro results to the clinical situation. Following addition of antibiotics to the vessel containing the bacteria, the desired half-life is determined by a pump-generated flow of antibiotic-free medium through the vessel.

Several dynamic models have been designed [6] but in recent years the hollow-fiber infection model has become predominant [11]. In this assay, experiments are performed using cartridges containing thousands of fibers that are permeable to smaller molecules. While the bacteria are trapped in the inner compartment, antibiotic molecules can enter from the fiber core to the bacterial compartment. Repeated sampling is possible for monitoring bacterial growth and emerging resistant populations. An advantage of the hollow-fiber infection model is the low risk for contamination and technical failure, enabling extended experiments of 7–21 days or longer to detect late-onset bacterial regrowth and emergence of resistance. Drawbacks include the high cost compared with other in vitro models and the high surface-to-volume ratio that could result in extensive drug binding and formation of biofilm, which greatly reduces antibiotic susceptibility. Of note, the working volume is usually substantially higher (e.g., 100-fold) in dynamic time-kill assays compared with MIC determination and static time-kill methods. Consequently, when similar starting inocula are used, the total number of bacteria will be higher in the dynamic models and emergence of resistant subpopulations is thus more likely to be observed during experiments.

3. Animal infection models

The goal of animal infection model efficacy studies is to identify potentially efficacious dosing regimens. Although the selection of the infection model is ideally based on the target indication, feasibility and animal ethical considerations are also important. Thus, the neutropenic thigh and lung infection models in mice are most often used [2,12].

3.1. Commonly used animal infection models

Thigh infections, which mimic soft-tissue infections in humans, are induced by injecting a bacterial suspension directly into the thigh muscle. The advantage of this infection model is its simplicity and low variability within and between studies, enabling small group sizes, which is preferred for ethical reasons. On the other hand, this is not a clinically relevant model as soft-tissue infections are less prevalent than other infection types. Nonetheless, it is an indispensable tool for generating the PK/PD data required to proceed with clinical trials [13,14].

The lung infection model is suitable for evaluating compounds targeting pneumonia-causing bacterial species. This model mimics the natural route of infection and to some extent the pathophysiological and phenotypic characteristics seen in pneumonia in humans. Mice are inoculated by depositing bacteria directly into the lung. This can be performed by various methods; the least invasive and technically easiest is to place a droplet of bacteria on the nares of the mouse and let the mouse inhale it. Other methods use direct inoculation of bacteria into the lung, either by intratracheal instillation or endotracheal inoculation.

Finally, peritonitis/sepsis models and complicated urinary tract infection models are clinically relevant options for severe and invasive disease that are considered helpful for early “go/no-go” decisions in preclinical development of antibiotics [15,16] as these are common target indications and could potentially also be used for PK/PD studies.

3.2. Study design and data analysis

Dose-fractionation studies over a 24-h period are performed to correlate the efficacy to one of the three PK/PD indices (peak concentration [Cmax] to MIC ratio, area-under-the-curve [AUC] to MIC ratio or time above MIC [T>MIC]) and to set the PD target [13]. The
primary endpoint is reduction in CFU counts at the site of infection. Thus, it is critically important when developing and using animal models of infection to accurately determine the bacterial dose given to each animal, to keep this consistent between experiments, and to monitor CFU counts over the disease course. Optimally a 1- or 2-log_{10} drop in bacterial loads over a 24-h period should be obtained with a successful antibiotic treatment [17]. Drug exposure data from the entire dose range used in the PD studies should be generated in animals under the same conditions as the PD studies because infection may alter PK.

4. Pharmacokinetic and pharmacokinetic/pharmacodynamic modeling

PK and PK/PD modeling summarizes preclinical or clinical data using mathematical functions. Objectives can be to test alternative hypotheses regarding concentration-effect relationships, to translate drug effects observed in preclinical experiments to the clinical situation while taking species differences into account, and to define the most informative study designs based on available knowledge [18]. A strength of non-linear mixed effects modeling is that variability at different levels, e.g., between-patient and day-to-day variability within patients, can be separated from residual variability. The developed model can be applied to perform simulations to test “what-if” scenarios.

4.1. Modeling procedure

PK/PD models are typically developed using data from in vitro time-kill studies from which CFU counts are available for a range of antibiotic concentrations and time points. Ideally, all counts from replicate experiments and dilutions are entered as separate data points in the dataset, rather than medians or means, to best acknowledge experimental variability. For PK modeling, data will include dosing history and administration route, measured plasma concentrations, and sampling time points. PK/PD models for animal data are still uncommon [19,20], but should include similar data.

During model building, different model structures are fitted to the dataset. The software (e.g., NONMEM, Monolix, Pmetrics) searches iteratively to find the parameters that best fit observed data. For PK/PD models, parameters may describe bacterial growth rate or maximal killing rate by drug, whereas for PK models, clearances and volumes are estimated. The model with the best fit, given the number of parameters included in the model, will be chosen as the final model. Mechanistic and biological plausibility in relation to information available in the literature, as well as model simulation capacity, are also common criteria for model selection.

Once developed, a model can be adapted or extended with new data. For development of a new compound, it is useful to proceed stepwise, e.g., (i) develop a model based on data from experiments with static concentrations, (ii) use the model to design experiments with dynamic concentrations, (iii) update the model, if needed, based on generated data from dynamic experiments, and then repeat steps (ii) and (iii) for animal data. Extensions of the model may be made with information on different strains or antibiotic combinations. Particularly for the latter, it quickly becomes experimentally infeasible to explore a complete matrix of concentrations, and modeling can help to quantify the interactions as concentrations vary with time.

4.2. Model evaluation: the visual predictive check

The gold standard for evaluating a model consists of simulation-based diagnostics and visual predictive checks (VPCs) [20]. To generate a VPC, a large number of datasets (e.g., 200-1000) are simulated from the developed model and its parameters using the original dataset structure as a template, i.e., the observed CFU counts or plasma drug concentrations are replaced with simulated ones. The median and percentiles of the simulated data are then compared with the original data (internal validation) to explore whether the original data could have arisen from the model, i.e., whether the model has good simulation capacity.

4.3. Model application: Optimize human dosing and improve study design

Once a model is established it can be applied to explore different dosing scenarios. For example, it is common to perform simulations from a population PK model to generate concentration-time profiles for a virtual patient population, and evaluate, for a range of MICs, how large is the proportion of patients that are expected to reach the PD target. These so-called probability of target attainment (PTA) simulations are frequently used to set clinical susceptibility breakpoints [3]. A population PK model can also be coupled to a PK/PD model describing bacterial counts over time for the simulations, and thereby make fewer assumptions on the translational capability of the PD target. Different dosing strategies can be compared to select the most promising one for clinical studies, and additional factors, such as risk of resistance development and toxicity, can be considered. Moreover, the models can be used to define when samples should be drawn to provide most information, which is particularly meaningful in larger studies where fewer samples per patient are possible.

5. Correlation with clinical pharmacodynamic data: Limitations of in vitro, animal, and modeling data

In vitro and animal infection models provide a wealth of microbiological susceptibility and toxicity data before an antibiotic is tested in humans. Indeed, data from these studies are required for entering first-in-human studies and, after clinical development, to obtain market approval by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Indeed, many PK/PD targets derived from preclinical data have been confirmed in human studies [21–23]. Yet preclinical models possess inherent limitations. In vitro data are generally likely to correlate with microbiological efficacy in humans provided there is a similar level of bacterial growth and exposure to the antibiotic (i.e., to its concentration over a similar period of time) at the in vivo site of infection. As discussed below, however, this cannot always be achieved. PK models derived from animal data are employed to predict human PK and animal infection models are applied to understand exposure-response effects in vivo as development moves closer to human testing. Here too, the complexity and particularities of the human host cannot always be predicted.

5.1. Limitations of in vitro models

There are many potential sources of discrepancy between in vitro and in vivo data. The absence of a functioning immune system is an inherent limitation to all in vitro models. Although some data on the antibacterial effects of neutrophils have emerged from animal models [24–26], these effects are not typically taken into account in the interpretation of data. Therefore, the clinical implications of phenomena observed in vitro, such as regrowth of persisting, non-resistant bacteria or selection of resistant subpopulations, remain uncertain. Conversely, bacterial persistence may not be captured in vitro, making laboratory results potentially incongruent with clinical efficacy studies assessing antibiotics with a mechanism of action that relies on active bacterial replication [27].
Bacterial and antibiotic concentrations typically mimic a high bacterial load (6–8 log_{10} CFU/mL) and unbound blood concentrations, while a range of bacterial densities may be present in the infected tissue and the drug concentration-time profile can differ.

Moreover, bacteria are normally cultivated in vitro in nutrient culture media at controlled temperature and pH whereas physiological growth conditions in vivo can be less favorable. Some in vitro models enable study of antibacterial effects in the presence of biofilms [28], which greatly impede the activity of antibiotics. These models, however, cannot exhaustively simulate and address all the vagaries of a dynamic in vivo infection, such as fluctuating (and increasing) bacterial loads and local environmental factors, including host alterations in iron sequestration and storage. Of note, rifampin has been shown to efficiently penetrate biofilms produced by staphylococci [29], but despite years of its adjunctive use in clinical infections has not been convincingly linked to superior clinical outcomes [30].

5.2. Limitations of animal infection models

Data from animal infection models may also be discordant with those observed in human studies. A major limitation of animal infection models is the variability among species in antibiotic PK, susceptibility or resistance to various infections, and drug toxicity. For example, in addition to size and enzyme differences affecting drug clearance, pH-dependent solubility may differ across species and consequently affect the rate and extent of absorption [31]. Perhaps the most notable example of an animal infection model paradoxical effect is that of penicillin. Many question why Alexander Fleming did not immediately champion development of penicillin for use in humans after observing its antibacterial effects in vitro: he was discouraged by a lapine model that indicated, erroneously, that elements in the animal’s blood were immediately inactivating the antibiotic [32]. Only years later, when Florey and Chain began using murine models, did research towards clinical applications gather momentum [33]. Due to the species differences in absorption, distribution, metabolism, and excretion of a given drug, extrapolations from animals should be performed with caution.

5.3. Limitations of mathematical models and prospective simulations

A mathematical model is limited by the data from which it was derived and the assumptions made in its construction. Even if a model has a good VPC, expanding beyond the conditions used to develop the model should be done with caution. Moreover, in PTA simulations, it is the PK profiles that will vary for each simulated patient, while the target is typically fixed and assumed to be correct. It is therefore important to understand the uncertainty in the target, which is typically derived from animal models and corresponds to a PK/PD index magnitude that results in 1- or 2-log_{10} CFU/mL kill, for example. Using targets that are based on time-course data and not summaries of the PK-profile (Cmax, AUC, T>MIC) and summary PD (MIC) data will likely expand in the future. As clinical data are collected, the targets could be changed to clinically-derived ones that may expand beyond drug exposure and MICs.

6. Conclusions and future perspectives

In vitro and animal models are essential for early evaluation of microbiological effects of new antibiotic treatments and their selection among candidate compounds. Data derived from preclinical studies are used to establish clinical breakpoints and many PK/PD targets have been confirmed in human studies. Through structured and iterative processes, PK/PD modeling integrates in vitro and animal data with human population PK to optimize dosing and inform future study design. All these approaches will remain essential given the workload, costs, and often low feasibility of adequately powered randomized clinical trials tailored to specific patient populations and pathogens. Moreover, they will continue to support clinical decision-making for situations in which patients do not fit into the standard template, i.e., those with varying PK or rare or difficult-to-treat pathogens.

More work is needed to improve the quality and external validity of preclinical studies, including more standardized methodological approaches. Efforts are underway, e.g., the Innovative Medicines Initiative’s recently launched Collaboration for Prevention and Treatment of Multi-Drug Resistant Bacterial Infections (COMBINE) [34], which aims to develop standardized protocols for the conduct and translation of rodent infection model. Optimizing available translational PK/PD tools has become increasingly important as we rely more and more on non-clinical data to predict successful treatment regimens. In clinical trials, PK/PD should be more routinely and systematically assessed, e.g., by characterization of causative pathogens and analyses of microbiological and clinical outcomes stratified by patient PK profiles, mode of administration (such as intermittent vs. continuous or prolonged infusion) and standard treatment vs. therapy guided by therapeutic drug monitoring [35,36].

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