BIOCHEMICAL PHARMACOLOGY 71 (2006) 1057–1065

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The role of pharmacodynamic research in the assessment and development of new antibacterial drugs

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ARTICLE INFO

Article history:
Received 8 September 2005
Accepted 24 October 2005

Keywords:
Antibacterial
Development
Pharmacodynamics
IVPM
Animal Models

ABSTRACT

Antibacterial resistance continues to increase world wide, with some bacterial pathogens exhibiting resistance to virtually all available drugs. As the plague of antibacterial resistance continues to grow and create serious therapeutic problems, it is essential that the development of new antibacterial agents continue. Pharmacodynamic research plays an important role in the development of new antibacterial agents, as pharmacodynamic data can help define the clinical potential of a new drug and identify the strengths and weaknesses in comparison to other drugs already on the market. Furthermore, pharmacodynamic experiments can help focus the clinical phases of drug development by providing key information on the pharmacodynamic parameters that influence efficacy and the pharmacodynamic targets that should be achieved to optimize clinical success. Characterization of these pharmacodynamic properties for a new drug in development can help direct the design of the best dose and dosing strategy for clinical trials. This review will focus on the tools, methods, and strategies used to characterize the pharmacodynamics of antibacterial agents and aide in their development for clinical use.

1. Introduction

Early in the process of antibacterial development, it is important to define the interactions of a new compound with potential bacterial targets. Initially, this process involves the establishment of the drug's spectrum of activity and potency using susceptibility assays. Standard susceptibility assays such as broth dilution, agar dilution and Etest provide a quantitative measurement of a drug's potency, and this potency is defined as the minimum inhibitory concentration (MIC), or the lowest concentration of drug assayed that prevents a bacterial inoculum from growing to visibly detectable levels. Although MIC data are valuable pieces of information that help define the therapeutic potential of a new antibacterial agent, MICs tell us nothing about (1) whether an antibacterial agent is bacteriostatic or bactericidal, (2) whether bactericidal activity is concentration-dependent or time-dependent, (3) the rate of bacterial killing, (4) whether the drug exhibits a post-antibiotic effect when levels fall below the MIC, (5) what parameter(s) of drug exposure most influence clinical efficacy, and (6) what pharmacodynamic target(s) should be reached to optimize therapy. It is questions such as these that form the foundation of antibacterial pharmacodynamic research. Using the pharmacodynamic models and methodologies discussed in this review, pharmacodynamic experiments can be employed early in the developmental process to provide greater insight into the therapeutic potential of a new antibacterial agent, and later in development to help direct the design of optimum doses and dosing strategies for clinical trials.

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0006-2952/$ – see front matter
doi:10.1016/j.bcp.2005.10.038

2. Pharmacodynamic research tools and methods

Quantitative MIC assays provide an important measure of drug potency and spectrum of activity. However, by their design, MIC assays only measure the ability of a static concentration of drug to inhibit the visible growth of bacteria over an established period of time (approximately 18–24 h). These assays do not distinguish between bacteriostatic and bactericidal drugs, as both can keep a bacterial culture below the limit of visible detection and exhibit exactly the same MIC. For the treatment of some infections, however, a bactericidal drug could have a decided therapeutic advantage over a bacteriostatic drug, even if they exhibit the same potency (MIC). Although broth dilution assays can be extended to provide information on bacterial killing, i.e. minimum bactericidal concentration (MBC), these data are still limited in their scope. With this in mind, pharmacodynamic methods have been developed to better define the antibacterial and clinical characteristics of antibacterial agents.

2.1. *Static time-kill assay*

The experimental design of static time-kill assays is very similar to that of MIC assays, with cultures of bacteria being exposed to static concentrations of an antibacterial agent over a predetermined period time. The concentrations of antibiotic evaluated are typically equal to the MIC or at two-fold increments above the MIC. However, in contrast to MIC assays, actual measurements of viable counts are performed at multiple time intervals during drug exposure, and changes in viable bacterial counts are evaluated quantitatively over time. The primary advantage of static time-kill assays over other pharmacodynamic methods is their relative simplicity and ease of execution. However, the exposure of bacterial cultures to static concentrations of antibiotic lacks the clinical relevance of other models described below. Specifically, bacteria are not exposed to the constantly changing concentrations of antibiotic that they would encounter during the treatment of many infections, i.e. increasing antibiotic concentrations after dosing and decreasing concentrations as antibiotic is metabolized and/or eliminated. To provide more clinically relevant pharmacodynamic evaluations, some investigators use in vitro pharmacokinetic models (IVPM) to expose bacteria to simulated pharmacokinetic profiles of antibiotics.

2.2. *One-compartment IVPM*

The basic design of a one-compartment IVPM is shown in Fig. 1. In this model, bacteria are inoculated into growth media in the central reservoir, and peak concentrations of antibiotic are dosed into the central reservoir with the bacteria. Elimination pharmacokinetics are then simulated by a process of dilution and elimination of drug in the central reservoir. Using peristaltic pumps, drug-free media is pumped from a dilution reservoir into the central reservoir while media containing drug and bacteria is pumped at the same rate from the central reservoir into the elimination reservoir. The rate at which drug is diluted and eliminated from the central reservoir is defined by the flow rate of the two peristaltic pumps, as determined using the following equation:

$$\mathrm{Cl} = (\ln 2)(V_c/T_{1/2})$$

where Cl is the clearance or flow rate of the peristaltic pumps, V_c the volume of media and culture in the central reservoir, and $T_{1/2}$ is the elimination half-life target.

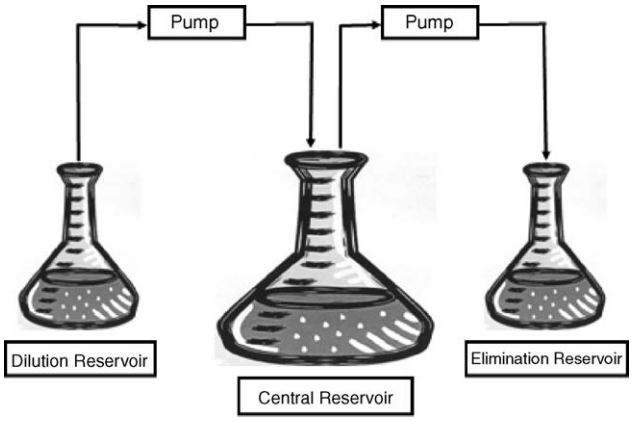

Fig. 1 – Schematic representation of a one-compartment IVPM. Peak concentrations of antibiotic are dosed into the central reservoir of the IVPM which also contains the bacterial culture in growth media. Using peristaltic pumps and tubing, antibiotic is eliminated from the central reservoir by the addition of drug-free broth from the diluent reservoir and elimination of drug-containing broth into the elimination reservoir. The flow rate of the pumps is established using an equation for mono-exponential decline to simulate virtually any elimination half-life desired.

The most important advantage of a one-compartment IVPM is the ability to evaluate pharmacodynamic interactions in an environment of changing drug concentrations that mimic the pharmacokinetics of the antibacterial in humans. Furthermore, the versatility of this model allows for the simulation of virtually any desired elimination half-life since the central reservoir volume and flow-rate capacity of the peristaltic pumps are the only limiting parameters. As will be discussed later, the versatility of one-compartment IVPM and two-compartment IVPM (described below) also allows for a more isolated evaluation of the impact of key pharmacodynamic parameters (C_{max}/MIC, AUC/MIC, etc.) on antibacterial activity. This versatility is not easily provided by animal models of infection, where pharmacokinetic parameters are more intimately linked to each other.

The primary disadvantage of a one-compartment IVPM is the dilution and elimination of bacteria while simulating the desired elimination half-life. Since the key to pharmacodynamic evaluations in IVPMs is the measurement of changing viable counts, the dilution and elimination of viable bacteria from the central reservoir can create a problem. This problem is especially evident in studies where the elimination half-life is short and dilution/elimination is rapid. In an attempt to account for the artificial loss of viable bacteria during elimination, two groups of investigators have developed and

refined differential equations that can be included in the data analysis [1,2]. However, another approach used to circumvent this problem is to study pharmacodynamics in two-compartment IVPMs.

2.3. Two-compartment IVPM

The basic design of a two-compartment IVPM is very similar to that of a one-compartment IVPM, except that bacterial cultures are contained within a peripheral compartment that is physically separated from the central reservoir where the antibacterial is dosed and eliminated. Some investigators use hollow-fiber cartridges as an integral part of two-compartment IVPMs, and a general schematic of a two-compartment model with hollow-fiber cartridges is shown in Fig. 2. The concept of using hollow-fiber systems to create a two-compartment pharmacodynamic model was first introduced by Zinner et al. [3] and Blaser et al. [4] in the 1980s, and has since been refined as hollow-fiber technology progresses. Bacterial cultures are inoculated into the extra-capillary space of the hollow-fiber cartridge (peripheral compartment) and antibiotic is dosed into the central reservoir. Peristaltic pumps direct the drug-containing media through the hollow fibers of the cartridge and back into the central reservoir using a continuous loop of silicone tubing. As drug passes through the lumen of the hollow fibers, it can diffuse through pores (10–30,000 molecular weight cut-off) in the fiber walls and enter into the peripheral compartment where the bacterial culture is contained. Drug continues to diffuse from the central compartment (fiber lumens) into the peripheral compartment until an equilibrium in drug concentration is established across the fiber walls. With the hollow-fiber technology available today, peak concentrations of antibiotic can be achieved in the peripheral compartment within 30 min of dosing into the central reservoir and starting the peristaltic pumps. Once peak concentrations have been achieved within the peripheral compartment, antibiotic is diluted and eliminated from the central reservoir using the same methodology described for the one-compartment IVPM. As drug concentrations decline in the central reservoir, antibiotic begins to defuse from the peripheral compartment back into the central compartment to maintain equilibrium. Since bacterial cells are too large to pass through the pores in the fiber walls, bacterial cultures in the peripheral compartment of the hollow-fiber cartridges can be exposed to pharmacokinetically relevant changes in antibiotic concentrations without actually disrupting the bacterial culture itself. This is just one advantage of using two-compartment IVPMs. Another important advantage shared by all IVPMs is the lack of host defenses that can interfere with direct evaluation of interactions between an antibacterial and its bacterial target. Although immunocompromised animal models can be used (i.e. neutropenic mouse model), there are still many other pathogen-host defense interactions that can inhibit and/or kill bacterial cells. In contrast, one- and two-compartment IVPMs allow for a more direct assessment of antibacterial pharmacodynamics in the absence of host defenses, and in some ways provide for analysis of a drug's pharmacodynamic activity in the worst-case scenario, with no help from host defenses.

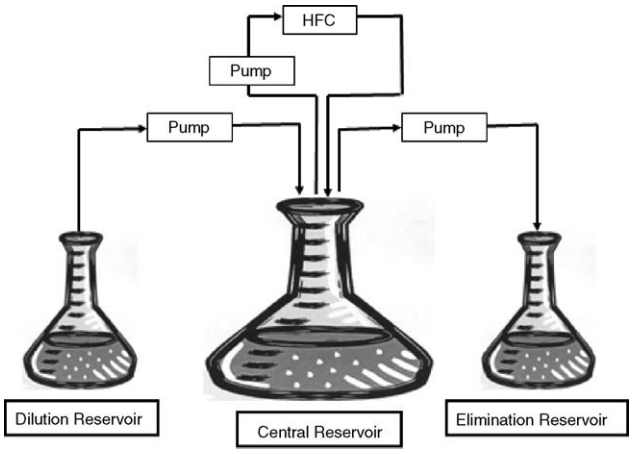

Fig. 2 – Schematic representation of a two-compartment IVPM. Peak concentrations of antibiotic are dosed into the central reservoir containing sterile growth media. Using peristaltic pumps and tubing, drug-containing media is pumped through the lumens of hollow fibers in the hollow-fiber cartridge (HFC) and back into the central reservoir. Pores in the fiber walls allow antibiotic to diffuse freely from the lumen of the hollow fibers into the peripheral compartment of the HFC where the bacterial culture has been inoculated. Antibiotic is eliminated from the central reservoir using the same methodology described for the one-compartment IVPM in Fig. 1. As antibiotic concentrations in the central reservoir and hollow-fiber lumens decrease, antibiotic concentrations within the peripheral compartment also decrease as drug diffuses back across the fiber walls to maintain equilibrium between the two compartments. The exclusion size of the pores in the fiber walls keeps the bacterial cells from escaping the peripheral compartment and entering the central reservoir.

2.4. Animal models of infection

IVPMs and static time-kill assays provide excellent platforms for analyzing antibacterial pharmacodynamics. However, they are still in vitro methodologies and lack the complex environment associated with animal models of infections or ultimately human infections. Animal infection models have been used to evaluate antibacterial pharmacodynamics since the 1950s when Eagle et al. first reported that the efficacy of penicillin for the treatment of streptococcal infections was dependent upon the length of time drug levels remained above the MIC [5,6]. There are several variations of animal infection models used for pharmacodynamic research, including pneumonia, peritonitis/septicemia, meningitis, endocarditis, and thigh infection models (reviewed in [7]). Although there are still important differences between these animal models of infection and the natural infections in humans, pharmacodynamic studies in animal models provide unique advantages that cannot be realized with the IVPMs. Some of the advantages of animal models over IVPMs include (1) the ability to evaluate clinical efficacy in addition to antibacterial pharmacodynamics, (2) the ability to evaluate the cooperative effects of antibacterial therapy and comprehensive host

defenses, and (3) the ability to assess the impact of antibacterial binding to serum proteins. For many IVPMs, the pharmacokinetics of the drugs are adjusted to account for measured protein binding in human serum, rather than including actual protein supplements into the media. While there are unique advantages associated with animal models of infections, they also present their share of challenges. The inability to truly differentiate between bacterial–antibacterial interactions and host defense–bacterial interactions has already been mentioned. Another important challenge is the significant differences in antibacterial pharmacokinetics in small laboratory animals compared to humans [7,8]. Unless pharmacokinetic differences are accounted for in experimental design, pharmacodynamic data from animal models may grossly underestimate the potential of an antibacterial agent for the treatment of human infections. One approach used to address this problem is to increase the number and frequency of doses. Another approach is to alter the elimination of the antibacterial agents after dosing. Depending upon the chemical properties of the drug being studied and method of excretion, this goal can be achieved through either the introduction of temporary kidney dysfunction with a nephrotoxic agent (i.e. uranyl nitrate) [9], or co-administration of a compound that will compete with the antibacterial agent for excretion, i.e. probenicid with penicillins [10].

3. Measured endpoints for pharmacodynamic studies

Just as there are many different types of models used for pharmacodynamic research, there are also numerous endpoints which can be measured during pharmacodynamic experiments. A summary of these endpoints is provided in Table 1.

3.1. *Rate and extent of bacterial killing*

Regardless of whether experiments are performed in vitro or with animal models of infection, measuring changes in viable bacterial counts over time is a straightforward method of evaluating pharmacodynamic interactions. However, in comparison to IVPMs, animal infection models are more versatile for this approach, allowing for measurements within different body fluids (blood and bronchoaveolar lavage fluid) or tissues (lung and thigh). The first endpoint which can be evaluated is the ability of a bactericidal drug to achieve at least three logs of significant killing, in contrast to bacteriostatic drugs which just inhibit cellular growth. Historically, a three-log reduction in viable counts has been considered the minimum response required to characterize as significant bactericidal activity. Secondly, bactericidal drugs can be further differentiated from each other by their rates of bacterial killing. The time required to achieve the three logs of significant killing (99.9% kill) is the most commonly used endpoint for comparing initial rates of bactericidal activity. However, depending upon the extent of bacterial killing, another endpoint may be the time required to achieve bacterial eradication from the model, i.e. decrease in viable counts below the limit of detection. Finally, bactericidal activity can also be measured in terms of the extent of bacterial killing. This endpoint can be measured as the net change in viable counts at a specific time point (end of a dose interval). In addition, some investigators use the area under

Table 1 – Pharmacodynamic endpoints for evaluation of antibacterial agents

Pharmacodynamic endpoint	Description of endpoint
Extent of bacterial killing	
Time to 99.9% kill	Time required for an antibacterial drug to kill 99.9% of starting inoculum (decrease viable counts three logs). Three logs of bacterial killing is considered the minimum for significant bactericidal activity. This endpoint allows for evaluation of the initial rate of killing
Time to eradication	Time required for an antibacterial drug to decrease viable counts below the level of detection of the plating methodology used. This endpoint allows for evaluation of both rate of killing as well as overall extent of killing
Extent of kill	When eradication is not achieved, another endpoint can be to measure the extent of killing at specific time points, i.e. end of dosing cycles
Area under bactericidal curve	Measure of the extent of antibacterial effect through evaluation of the total bacterial burden over time
Inoculum regrowth and emergence of resistance	
Timing of regrowth	Analysis of the time regrowth initiates in relation to the time antibacterial drug levels fall below the MIC. Relates to PAE and PA-SME assessments
PAE	The continuation of antibacterial activity (stasis or killing) after drug has been removed from the environment
PA-SME	Extension of the PAE by subinhibitory concentrations of antibacterial drugs
Emergence of resistance	Outgrowth of mutant subpopulations of bacteria exhibiting significantly decreased susceptibility to the antibacterial drug of interest. Evaluated by plating samples on/in agar containing the antibacterial agent a concentration $\geq$ 4-fold above the MIC
Animal model endpoints	
Protection from lethal infection	Dose of antibacterial drug that protects animals from a lethal infection
Pathological changes	Effect of antibacterial agent on pathology to tissues specific for the animal infection model, i.e. tissue damage, inflammation, necrosis. Subjective measurement of the extent of pathology
Physiological changes	Effect of antibacterial agent on physiological hallmarks of an infection, i.e. fever, weight changes, blood chemistry profiles, inflammatory and immune mediators

the bactericidal curve (viable bacteria-versus-time) as a measure of the extent of antibacterial activity [11,12].

3.2. Inoculum regrowth and emergence of resistance

It is not uncommon to observe inoculum regrowth during the course of pharmacodynamic experiments. In some experiments, inoculum regrowth can be directly related to loss of antibacterial activity as drug levels fall below the MIC. However, inoculum regrowth can also be associated with the outgrowth of mutant subpopulation(s) that exhibit significant decreases in their susceptibility to the antibiotic. The selection of resistance by an antibacterial agent is an important pharmacodynamic characteristic to evaluate, since this can impact the usefulness of the drug in clinical practice. Although selection of resistance can also be evaluated through exposure of bacterial cultures to static concentrations of drug (either subinhibitory or superinhibitory), IVPMs and animal models of infection allow for a more clinically relevant evaluation of this potential therapeutic problem. During pharmacodynamic experiments, emergence of resistance is evaluated by plating samples in/on growth media containing antibiotic at a concentration $\geq$4-fold above the MIC. The use of drug concentrations that are $\geq$4-fold above the MIC for detection of mutants is an important experimental parameter since a two-fold change in MIC is not considered significant with susceptibility assays. Colonies growing on drug-selection plates can be isolated and potential mutants analyzed for changes in susceptibility to the drug of interest, as well as unrelated antibacterial agents. In addition, molecular methods can be used to determine if an antibiotic has the propensity to select for specific mechanisms of resistance during therapy.

When resistance emerges during the course of therapy, it is typically associated with a stable mutational change affecting the susceptibility of the bacterial pathogen. However, it is not uncommon to observe reversible decreases in the efficacy of an antibacterial agent over multiple dosing cycles of a pharmacodynamic experiment. This phenomenon has been described as adaptive resistance, or the reversible decrease in susceptibility upon exposure of bacteria to an antibiotic. Adaptive resistance has been observed with ß-lactams [13,14], aminoglycoside [15–17], and fluoroquinolones [18,19], and the decreased efficacy of second and subsequent doses results in either net bacteriostasis or gradual net increases in viable counts over those dosing intervals.

3.3. Post-antibiotic effects

When the regrowth of a bacterial culture is related to loss of inhibitory drug concentrations, rather than emergence of resistance, it is important to evaluate the timing of regrowth as it relates to the time drug levels fall below the MIC. When a substantial extension of antibacterial activity (stasis or continued killing) is observed after inhibitory drug concentrations have been lost, this phenomenon is referred to as the post-antibiotic effect or PAE [20]. The PAE concept was first described with penicillin against streptococci/staphylococci [21,22]. Technically, PAEs refer most specifically to experiments where antibacterial drug is removed abruptly through centrifugation-washing, use of inactivating enzymes, etc. However, during the course of treating bacterial cultures in IVPMs or actual infections in animal models/patients, the removal of antibacterial drug is more gradual and there are extended periods of time when sub-inhibitory concentrations remain in the environment. Pharmacodynamic studies have shown that sub-MIC concentrations of an antibacterial drug can prolong the PAE [14,23–25], and this phenomenon has been designated as the post-antibiotic sub-MIC effect (PA-SME).

Another consideration for PAE measurements is the added effect of host defenses, particularly phagocytic white blood cells. Pharmacodynamic experiments have demonstrated that bacteria experiencing PAEs or PAE-SMEs are more susceptible to phagocytosis and intracellular killing. Therefore, it is not surpising that these endpoints are extended in experimental models where antibacterial phagocytic cells are present [26,27].

3.4. Endpoints specific for animal models of infections

Animal models of infection offer the closest simulation of actual infections in humans. Furthermore, there are pharmacodynamic/efficacy endpoints associated with animal models that are not measurable with in vitro models. These endpoints include (1) protection of animals from lethal infection, (2) impact of treatment on the physiological effects of an infection such as temperature changes, weight loss, changes in blood chemistry profiles, changes in levels of inflammatory mediators, and (3) prevention of pathological hallmarks of the infection including tissue damage, inflammation, necrosis, etc. Of these endpoints, the protection of animals from lethal infection and monitoring effects on the physiological effects of infection are the most straightforward. Evaluating the effects of an antibacterial agent on pathological hallmarks of disease can be more subjective and difficult to interpret.

Although measuring protective efficacy is straightforward in theory, this experimental approach offers unique challenges that can make data analysis difficult. Selecting mortality as an endpoint increases the distress, discomfort, and/or pain of experimental animals, and steps must be taken to minimize these negative effects. This may require the euthanasia of animals that appear moribund to prevent any prolongation of their suffering, and these humane judgments can skew the data. In addition, the establishment of infections with some strains of bacteria may require excessively high numbers. Not only is there a risk that the high inocula will not correlate with the natural infections in humans, but the burden of bacteria injected may be rapidly lethal due to overstimulation of the inflammatory response and host defenses, i.e. endotoxin shock. Therefore, mortality may not always be the result of an established infection, but may be related to the size of the inoculum, making any analysis of antibacterial treatment effects difficult. All of these factors must be carefully considered when designing experiments in animals to ensure the validity of the data obtained from them.

4. Pharmacodynamic studies and analysis for antibacterial drug development

Once the spectrum and potency of an antibacterial agent have been defined by susceptibility assays, the pharmacodynamic

tools and endpoints discussed above can be used to more clearly define the antibacterial activity and clinical potential of a new drug. Pharmacodynamics studies can identify whether a new antibacterial agent is (1) a bacteriostatic or bactericidal drug (2) a concentration-dependent killer, or (3) a time-dependent bactericidal agent. Furthermore, pharmacodynamic studies can define the pharmacodynamic parameter(s) that are most closely linked to clinical efficacy and identify the minimum target that is required to optimize clinical efficacy. Taken together, these data can help focus clinical trials by aiding in the selection of optimum doses.

4.1. *Bactericidal versus bacteriostatic antibacterial activity*

As previously discussed, the data obtained from susceptibility assays measuring the MIC tell us nothing about the mechanism of antibacterial activity. One important characteristic to define is whether the mechanism of antibacterial activity involves significant killing of the bacterial target (bactericidal activity) or whether the drug reversibly inhibits bacterial growth (bacteriostatic activity). Although both bactericidal and bacteriostatic drugs can exhibit the same potency (MIC), a drug that exhibits significant bacterial killing is decidedly more appealing for the treatment of some infections. For example, bacterial killing by an antibiotic is usually warranted to optimize treatment of bacterial endocarditis [28,29]. Furthermore, an agent that kills the infecting bacterial pathogen is likely to provide a faster rate of bacterial eradication and resolution of clinical symptoms. Even among bactericidal drugs that exhibit different potencies and rates of killing, differences can be observed in the time to resolution of symptoms [30].

4.2. *Concentration-dependent versus time-dependent bacterial killing*

Once a new antibacterial has been characterized as bactericidal, the next characteristic to evaluate is whether the bactericidal activity is concentration-dependent or time-dependent. In reality, all bactericidal drugs are dependent upon concentration. However, for antibacterial agents like the fluoroquinolones and aminoglycosides, the rate of bacterial killing increases over a broad range of increasing drug concentrations above the MIC [26,31–35]. In contrast, β-lactam drugs exhibit their maximum rate of bacterial killing at approximately four times the MIC, and their pharmacodynamic activity is related more specifically to the time drug levels remain above the MIC [31,36–39].

Knowing whether a new antibacterial agent concentration-dependent or time-dependent in its killing is important for further assessment of its clinical potential. For example, with concentration-dependent drugs, evaluating the relationship between the drug's potency against a bacterial pathogen (MIC) and its pharmacokinetic profile (i.e. C_{max} levels) in humans can provide an early indication of how the drug compares to other concentration-dependent drugs on the market. The rationale for this analysis comes from pharmacodynamic data showing that increasing levels of exposure to concentration-dependent drugs correlate with increasing rates of and extent of bacterial killing [35,40,41]. Although this well-established pharmacodynamic principal comes from in vitro experimentation, the clinical relevance has been demonstrated. One of the best examples is the comparison of 750 and 500 mg doses of levofloxacin for the treatment of community-acquired pneumonia. The newer 750 mg dose of levofloxacin provides a more favorable pharmacokinetic profile in patients [42], and clinical data have suggested that the enhanced pharmacodynamic properties of the 750 mg dose provide a faster resolution of symptoms in patients with community-acquired pneumonia [43,44]. Therefore, if enhancing the pharmacokinetics of a drug can provide a faster clinical response, comparative analysis of the potency and pharmacodynamic properties of a new antibacterial agent to others on the market may provide important direction for future development and assessment.

5. Pharmacodynamic parameters influencing clinical efficacy of antibacterial agents

Integrating the potency (MIC) of a drug with its pharmacokinetics can provide a powerful tool for evaluating the pharmacodynamic potential of an antibacterial agent. After defining the key pharmacokinetic and pharmacodynamic parameters that influence clinical efficacy, strategies for defining these parameters and targets during the development of an antibacterial agent will be addressed.

5.1. *Pharmacokinetic and pharmacodynamic parameters*

Fig. 3 illustrates a simplified concentration-versus-time pharmacokinetic curve for an antibacterial agent, with an increase in drug concentrations to the peak or C_{max} concentration and subsequent decrease in drug concentrations as the drug is eliminated from the body. The AUC (shaded in grey) represents the area under the concentration-versus-time curve, or a measure of total drug delivered. Also represented in this figure is the potency of the antibacterial agent (MIC). The three key pharmacodynamic parameters that have been linked to the clinical efficacy of antibacterial agents are (1)

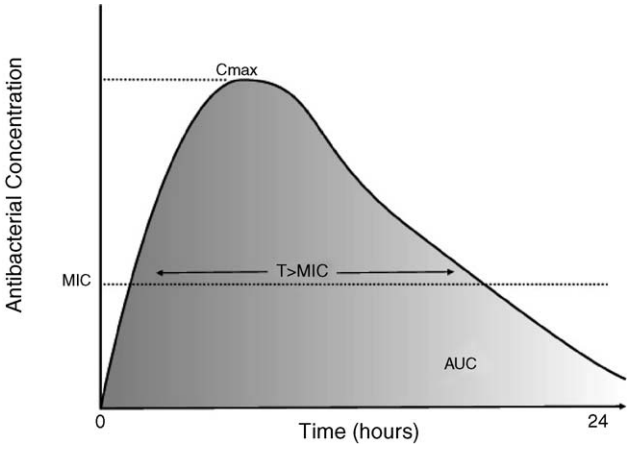

Fig. 3 – Antibacterial concentration vs. time graph illustrating key pharmacokinetic and pharmacodynamic paramaters.

C_{max}/MIC ratio, (2) AUC/MIC ratio, and (3) $T > MIC$ or time antibacterial concentrations exceed the MIC. As would be predicted, concentration-dependent antibacterial drugs are most influenced by parameters that relate drug exposure to potency, i.e. C_{max}/MIC and AUC/MIC. In contrast, time-dependent drugs are most influenced by $T > MIC$. Not only are there differences between antibacterials with respect to the influence of these pharmacodynamic parameter(s), but there are also differences in the minimum target that is required for optimum response. An indepth discussion of the pharmacodynamic targets required to optimize clinical efficacy is beyond the scope of this review. However, there are several other excellent reviews which address this topic [31,34–36,45–47]. For the development of a new antibacterial drug, the important issue is how to use pharmacodynamic experiments to dissect the drug and (1) determine which parameter most influences clinical efficacy, (2) identify what minimum target is required to optimize pharmacodynamics and clinical response, and (3) use this information to direct dose selection for clinical trials. To illustrate how pharmacodynamic experiments can be designed to address these questions, experimental strategies for analysis of a concentration-dependent bactericidal drug will be discussed below.

5.2. *Experimental strategy for identification of efficacy-linked pharmacodynamic parameter(s)*

Once a new antibacterial drug has been characterized as a concentration-dependent killer, the next step is to define whether its efficacy is linked most closely to C_{max}/MIC or AUC/MIC. Although this analysis may seem straightforward, these experiments can be challenging since all three of the key pharmacodynamic parameters are interdependent upon each other, especially in animal models and humans. For example, if the dose of an antibiotic is increased to provide higher C_{max} levels, a concomitant increase in the AUC and $T > MIC$ are also observed. Therefore the impact of C_{max}/MIC on efficacy cannot be directly evaluated by simply increasing the dose of an antibacterial agent while keeping the dosing interval constant. Rather, these questions are addressed through the design of dose-fractionation experiments, where the amount of drug delivered over 24 h (AUC_{24}) remains the same for each dosing regimen, but the size of each dose and dosing intervals are altered. An example of a dose-fractionation experimental design would be to give one experimental arm the highest dose delivered once daily. In a second experimental arm of the study, the once daily dose would be divided into two equivalent doses given twice daily at 12-h intervals. In a third experimental arm, the once daily dose would be divided into three equivalent doses given at 8-h intervals over 24 h. Therefore, for each arm of the experiment, the total amount of drug delivered over 24 h (AUC_{24}) would remain constant. However, as the once-daily dose is fractionated, the C_{max} achieved with the fractionated doses would decrease accordingly. If the antibacterial response or clinical efficacy is similar for all three regimens, this would suggest that AUC/MIC is the most important pharmacodynamic parameter since AUC/MIC remained constant for all regimens. In contrast, if significant differences in antibacterial response or clinical efficacy are observed between the regimens, these data would suggest that C_{max}/MIC is most important pharmacodynamic parameter.

5.3. *Experimental strategy for identification of optimum pharmacodynamic target*

Once the efficacy-linked pharmacodynamic parameter has been defined, the next step is to define what pharmacodynamic target(s) are required to optimize therapy against different bacterial pathogens. Because of their flexibility for simulating virtually any pharmacokinetic profile desired, the IVPMs are especially suited for this stage of pharmacodynamic evaluation. This point is highlighted by a recent study of the pharmacodynamics of gatifloxacin against *Streptococcus pneumoniae* [48]. The goal of this study was to define the minimum AUC/MIC required to optimize antibacterial activity, i.e. eradication of *S. pneumoniae* from an IVPM. To accomplish this goal, it was important to isolate AUC/MIC interactions away from C_{max}/MIC, since gatifloxacin is a fluoroquinolone that exhibits concentration-dependent bactericidal activity. Therefore, the experimental strategy for this study was to use a two-compartment IVPM with hollow-fiber cartridges to expose cultures of *S. pneumoniae* to C_{max}/MIC of 2–3, while simulating a range of AUC/MIC through alterations in the rate of gatifloxacin elimination from the model [48]. Since this strategy allowed for exposure of the *S. pneumoniae* to a range of AUC/MIC without any differences in C_{max}/MIC ratio, pharmacodynamic responses could be related specifically to the impact of AUC/MIC. Data from this study demonstrated that AUC/MIC of ≥ 27 were required to achieve eradication of the *S. pneumoniae* isolates from the IVPM, a pharmacodynamic endpoint that was not achieved when AUC/MIC were ≤ 24 [48].

6. Summary

Pharmacodynamic research is a powerful tool that can unlock the clinical potential of an antibacterial agent early in the developmental process. Not only can pharmacodynamic data identify the strengths/weaknesses and clinical potential of a new drug, but the identification of which pharmacodynamic parameter(s) influence clinical efficacy and what pharmacodynamic target(s) must be achieved to optimize clinical efficacy can help direct the design of doses for clinical phases of development. This concept was used to guide the clinical assessment and development of linezolid, where data from pharmacodynamic experiments in animals led to the design of the 600 mg twice-daily dosing regimen for phase III clinical trials [49,50]. As the need for new antibacterial agents increases with increasing threats of antibacterial resistance, pharmacodynamic research will play a central role in the process of developing and assessing new drugs for clinical use.

Acknowledgments

The author would like to thank Joy Nielsen and Phil Beagle for their artistic help in preparing the figures for this manuscript.

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