A Novel Approach to Pharmacodynamic Assessment of Antimicrobial Agents: New Insights to Dosing Regimen Design

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Abstract

Pharmacodynamic modeling has been increasingly used as a decision support tool to guide dosing regimen selection, both in the drug development and clinical settings. Killing by antimicrobial agents has been traditionally classified categorically as concentration-dependent (which would favor less fractionating regimes) or time-dependent (for which more frequent dosing is preferred). While intuitive and useful to explain empiric data, a more informative approach is necessary to provide a robust assessment of pharmacodynamic profiles in situations other than the extremes of the spectrum (e.g., agents which exhibit partial concentration-dependent killing). A quantitative approach to describe the interaction of an antimicrobial agent and a pathogen is proposed to fill this unmet need. A hypothetical antimicrobial agent with linear pharmacokinetics is used for illustrative purposes. A non-linear functional form (sigmoid Emax) of killing consisted of 3 parameters is used. Using different parameter values in conjunction with the relative growth rate of the pathogen and antimicrobial agent concentration ranges, various conventional pharmacodynamic surrogate indices (e.g., AUC/MIC, Cmax/MIC, %T>MIC) could be satisfactorily linked to outcomes. In addition, the dosing intensity represented by the average kill rate of a dosing regimen can be derived, which could be used for quantitative comparison. The relevance of our approach is further supported by experimental data from our previous investigations using a variety of gram-negative bacteria and antimicrobial agents (moxifloxacin, levofloxacin, gentamicin, amikacin and meropenem). The pharmacodynamic profiles of a wide range of antimicrobial agents can be assessed by a more flexible computational tool to support dosing selection.

Introduction

Microbial resistance is rising at an alarming rate, rendering many antimicrobial agents ineffective. There is an ever demanding need to develop new antimicrobial agents and optimize available agents to curb the rising resistance prevalence. There are experimental and clinical evidence that dosing exposure could have an impact on patient outcomes and the development of resistance [1,2]. As a result, pharmacodynamic modeling has been increasingly used as a decision support tool to guide dosing selection in new drug development [3].

Microbial killing by unbound antimicrobial agents has been traditionally classified categorically as concentration-dependent or time-dependent. The pharmacodynamics of many antimicrobial agents are well accepted to be linked to surrogate indices such as peak concentration (Cmax)/minimum inhibitory concentration (MIC), area under the concentration-time profile (AUC)/MIC or the proportion of dosing interval in which the concentration is above the MIC (%T>MIC) [4]. Dosing regimen design could be based on results from traditional dose fractionation studies [5–7]. As long as the pharmacokinetics of the antimicrobial agent is linear, the entire daily dose could be given once daily, half the daily dose given twice daily, one-third the daily dose given three times each day, etc. to achieve a similar daily AUC. Depending on the difference in outcomes observed (if any), different categorical pharmacodynamic characterization could be deduced. For instance, if the once-daily regimen is the most effective, Cmax/MIC would be most likely linked to outcomes. If the most frequent dosing regimen is found to be the most beneficial, %T>MIC [or minimum concentration (Cmin)/MIC] would likely be the most useful pharmacodynamic surrogate index predicting outcomes. If all the dosing regimens are similar, AUC/MIC would be deemed to be associated with outcomes. This traditional approach has been applied in a wide range of infection types, patient groups and duration of therapy [1]; heterogeneous bacterial populations at the infection site have also been considered [8].

While the dose fractionation design is intuitive and commonly used, there may be situations where such a categorical approach to pharmacodynamic assessment is overly restrictive. It is especially so with new drugs (or drug classes) with complex pharmacokinetics and pharmacodynamics. We propose an alternative approach to pharmacodynamic characterization of the interaction between an antimicrobial agent and a pathogen. Instead of relying on time-dependent endpoints such as Cmax or MIC, the time course of a
bacterial population when subjected to an antimicrobial agent exposure is captured by a dynamic mathematical model. The advantages of such a modeling approach have been reviewed previously [3,9]. The proposed pharmacodynamic characterization can be subsequently linked to a novel computational method to derive the dosing intensity of a dosing regimen, which would facilitate objective comparison of various dosing regimens [10]. To illustrate our approach in the clearest way possible, a hypothetic drug is used via a series carefully constructed computer simulations. The relevance of our approach is subsequently supported by application to several experimental datasets we have reported in the past.

**Methods**

**Basic assumptions**

A hypothetic drug is used for illustrative purposes. The pharmacokinetics of this drug is linear and characterized by a one-compartment intravenous bolus model. The volume of distribution of this drug is 20 liters, with an elimination half-life of 1 hour and negligible protein binding. A daily dose of 6000 mg can be given once daily (6000 mg q24h), twice daily (3000 mg q12h) or 4 times daily (1500 mg q6h). The respective serum concentration-time profiles and exposures achieved are as shown in Figure 1 and Table 1. In addition, the target pathogen is assumed to have a growth rate ($K_g$) of 1.0 h$^{-1}$ (microbial doubling time of approximately 42 minutes); resistance is not expected to incur a significant biofitness cost (no change in bacterial growth rate over time).

**General experimental design**

Details of the experimental setup have been reported previously [11–13]. Briefly, a heterogeneous bacterial inoculum (consists of multiple sub-populations associated with different susceptibility - a function of the total bacterial burden and the mutational frequency to resistance of the isolate being studied) is exposed to different and escalating drug concentrations; the drug concentration in each experiment is constant over time. Serial samples are taken from various flasks over 24 hours to determine viable bacterial burden by quantitative cultures. A dynamic mathematical model is fit to the data to derive the best-fit model parameter estimates. The growth rate of the target pathogen can be derived from placebo control experiments. Regrowth observed after an initial decline in bacterial burden is attributed to adaptation of the bacterial population under a selective pressure (i.e., enrichment of

Figure 1. Dose fractionation designs of an identical daily dose. doi:10.1371/journal.pcbi.1001043.g001
The bacterial population cannot be achieved within one dosing interval; a large killing rate shortly after dose administration is as important as a large killing rate towards the end of the dosing interval. The importance of the average kill rate (\(D\)) is that complete eradication of the entire bacterial population and prevention of resistance emergence will be observed, if \(D \geq K_k\) for the most resistant cell in the population. The preceding statement assumes that no physiological changes (e.g., induction of efflux pumps or beta-lactamases) take place in bacteria as a result of exposure to the antimicrobial agent. All simulations were performed using the ADAPT II program [20], and graphs were plotted with Mathematica 6.0 (Wolfram Research, Inc., Champaign, IL).

### Results

#### Pharmacodynamic profiles

Using the same structural form, three typical pharmacodynamic profiles can be shown (Figures 2–4). These distinct pharmacodynamic profiles are characterized by 3 parameter estimates and represent unique (extreme) situations. In reality, intermediate profiles (e.g., partially concentration dependent killing) are also possible and could be objectively described by these parameter estimates. The growth rate of the target pathogen is shown concurrently for comparison. Finally, the concentration ranges of antimicrobial agent attained by different dosing regimens are also shown in each situation.

In Figure 2, the maximal killing rate is significantly higher than the growth rate of the target pathogen. The concentration-effect relationship is also fairly linear in the concentration ranges achieved by all the dosing regimens. Therefore, attaining a high concentration for a brief period of time would not be much more advantageous, as compared to lower concentrations for a more prolonged time frame. As a result, dosing frequency is not expected to have a huge impact of the overall bactericidal activity, as long as the daily dose is kept constant. Using conventional nomenclature, this pharmacodynamic profile is concentration dependent killing, and AUC/MIC would be considered the most important. This pattern is consistent with our experience with data reported for moxifloxacin [21] and levofloxacin against *Escherichia coli* (unpublished data).

In Figure 3, the maximal killing rate is still significantly higher than the growth rate of the target pathogen. However, the concentration-effect relationship is non-linear in the concentration range achieved by the dosing regimens. The killing rate rises sharply beyond a certain threshold (i.e., approximately 75 mg/l - the peak concentration of the q6h dosing regimen). A dosing regimen attaining a concentration beyond this threshold could encounter a more than proportional increase in killing rate, even for a brief period of time. Consequently, less frequent dosing (to achieve a concentration transiently above the threshold) is expected to result in a greater overall bactericidal activity. Using conventional nomenclature, this pharmacodynamic profile is concentration dependent killing, and Cmax/MIC would be considered the most important.

An important point should be raised here. The above example is illustrated with only one daily dose (6000 mg daily). Using a qualitative (yes / no) outcome assessment (e.g., mortality, clinical cure, resistance suppression), different categorical interpretations may be arrived at with the same pharmacodynamic profile. To further exemplify this point, the average kill rate is graphed as a function of both daily dose and dosing interval, using a 3-dimensional surface response as detailed previously [22]. In addition, a cross sectional view is undertaken when the average kill rate...
rate equals to the growth rate of the pathogen ($D = K_g = 1.0 \text{ h}^{-1}$). When the average kill rate of a dosing regimen is greater than the growth rate of the target pathogen (i.e., $D > K_g$), resistance suppression is generally anticipated. On the other hand, if the average kill rate of a dosing regimen is less than the growth rate of the target pathogen (i.e., $D < K_g$), resistance amplification is expected over time. Given that AUC and Cmax are highly correlated in clinical or animal studies (where drug clearance cannot be easily modified), it may be difficult to discriminate whether AUC/MIC or Cmax/MIC is the pharmacodynamic variable most closely linked to outcomes. Our analysis revealed that there could be experimental evidence supporting both conclusions in the same antimicrobial agent-pathogen combination. The daily dose(s) to be examined in fractionation studies should also be carefully considered in the analysis. The dose exposure used may not be the same for different outcomes (e.g., survival, 1-log drop in microbial burden, or resistance suppression), and thus may have contributed (at least partially) to enthusiastic debates in the literature [23–26]. Other investigators have also reported different pharmacodynamic characterization at different dose levels of the same drug. Fractionation of the minocycline dose at the human dose equivalents showed no difference between once, twice, or three times a day dosing against Staphylococcus aureus (i.e., AUC/MIC was the most important). In contrast, fractionation of the dose with a static effect indicated that once daily dosing was superior (i.e., Cmax/MIC was the most important) [27]. In addition, these conflicting patterns are also consistent with our own experience examining the pharmacodynamics of the aminoglycosides. Using a dose fractionation design, there were data to support gentamicin AUC/MIC was the most important against Pseudomonas aeruginosa [11]; but amikacin Cmax/MIC appeared to be the most important against Acinetobacter baumannii [13].

Finally in Figure 4, the maximal killing rate is only slightly higher than the growth rate of the target pathogen. The concentration-effect relationship is non-linear in the concentration range achieved by the dosing regimens, but the threshold which the killing rate rises sharply (i.e., approximately 5 mg/l)
could be readily attained by all 3 dosing regimens. Under such circumstances, a transiently high concentration achieved in a less frequent dosing regimen does not translate to a higher kill rate, and killing is compromised when the concentration falls below the threshold for a prolonged period of time. Consequently, a more rational dosing strategy is to maintain concentrations above the threshold for as long as possible. Using conventional nomenclature, this pharmacodynamic profile is time dependent killing, and %T > MIC (or Cmin/MIC) would be considered the most important when the investigations are performed in the ‘critical’ daily dose range. In the extremes of the daily dose range (i.e., more than 14000 mg or less than 2000 mg daily), AUC/MIC could still be interpreted as an important surrogate linked to outcomes observed. This pattern is consistent with our experience with data reported for meropenem against *Pseudomonas aeruginosa* [28].

**Discussion**

In all 3 typical pharmacodynamic profiles described, the same structural form of killing rate was used (a sigmoid Emax model). Different profiles were simply reflected in the values of the model parameters, which could be derived from actual experimental data observed between an antimicrobial agent and a pathogen within a short timeframe (e.g., 24 hours). Of note, knowledge of specific mechanism(s) of resistance was not necessary as inputs. As shown above, an antimicrobial agent can be shown to exhibit both concentration and time-dependent killing, but the proposed model was flexible enough to describe different distinct pharmacodynamic profiles (and any intermediates in between).

Antimicrobial kill kinetics has been previously examined using a related approach. In one study, concentration dependency of bacterial killing was primarily attributed to the sigmoidicity
constant (i.e., \( H \) in our model) [29]. Drugs exhibiting concentration-dependent killing was associated with a low sigmoidicity constant. In our opinion, all drugs exhibit concentration-dependent killing in the concentration range corresponding to 20%–80% of maximal killing rate (regardless of the magnitude of sigmoidicity). It is equally important to consider \( K_k \) and \( C_{50} \), in order to get a complete and accurate pharmacodynamic assessment of an antimicrobial agent. As reviewed previously by Czock et al [30], the effect of the sigmoidicity constant could be influenced by the ratio of \( K_k / K_g \) (similar illustrations were also shown in Figures 3 and 4). In addition, whether such concentration-dependent killing is clinically relevant is further dependent on whether the concentration-dependent killing concentration range is achievable in humans with acceptable toxicity, potentially resulting in other categorical descriptions on a continuous spectrum such as “partially concentration-dependent” (where there is partial overlap of the concentration ranges) or “time-dependent” killing (where there is minimal overlap of the concentration ranges). Thus \( C_{50} \) is also not irrelevant as pointed out previously [30]. Consequently, we resorted to a more comprehensible approach by taking into consideration additional pertinent variables. We attempted to develop a more robust computational tool covering a wider spectrum of relevant scenarios in new drug development.

To extend these pharmacodynamic concepts previously reviewed, we put forth herein a novel concept to derive the dosing intensity of a dosing regimen by comparing the average killing rate to the growth rate of the target pathogen. A similar approach was proposed in linking pharmacokinetics to drug effects in circular / proliferative systems using the concept of reproduction minimum inhibitory concentration (RMIC) and equivalent effective constant concentration (ECC) [31]. As we have shown in Figure 2, in the unique case of linear concentration-effect relationship, the heuristics proposed previously (RMIC and ECC) would be identical. Dosing frequency is not expected to have a major impact of effectiveness, as long as the daily dose is kept constant (i.e., AUC/MIC is the most important). To highlight a common drawback in conventional modeling, our computational tool illustrated the pharmacokinetic / pharmacodynamic concepts via several theoretical and experimental case examples.

We believe our proposed approach would enhance the applicability of these concepts in drug development and clinical settings. Specifically, the pharmacodynamics of antimicrobial agents are characterized as a continuum (as opposed to discrete

Figure 4. Time dependent killing: %T>MIC most important. \( K_k = 4.0 \text{ h}^{-1} \), \( C_{50} = 10.0 \text{ mg/l} \), \( H = 4.0 \). Black solid line depicts the relationship between drug concentration and killing rate; black dotted line represents the microbial growth rate. Arrows below represent concentration ranges achieved with various dosing regimens (red – once daily; green – twice daily; blue – four times daily). White area depicts dosing regimens (combinations of daily dose and dosing interval which the average kill rate is >1.0 h\(^{-1}\)). Using a daily dose of 6000 mg, the average kill rates for different regimens are: 0.818 h\(^{-1}\) (q24h), 1.303 h\(^{-1}\) (q12h), and 1.950 h\(^{-1}\) (q6h).

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categories), allowing relatively simple numeric computational methods to be applied. The efficiency to objectively compare the effectiveness of a large number of dosing regimens (to be investigated in pre-clinical or clinical studies) would be improved as a result. Furthermore, we have also justified our rationale using supportive data derived under more clinically relevant experimental conditions (multiple sets of experimental data involving fluctuating drug concentrations over at least 72 hours, as shown in Table 2). Such an approach provides a fresh perspective on our understanding of antimicrobial agent pharmacodynamics; the new insights could resolve heated debates in the literature relating to which pharmacodynamic surrogate index is the most closely related to outcomes.

In this study, the mathematical model used was to characterize the behavior of a heterogeneous bacterial inoculum, which consists of multiple sub-populations associated with different susceptibility. This modeling approach (involving a time-variant parameter to account for adaptation of the bacterial population) would enable us to better capture regrowth and / or emergence of resistance over time. Nonetheless, the mathematical model is flexible and can be modified easily to accommodate a homogeneous inoculum by not allowing $G_{sp}$ (an index of bacterial population susceptibility) to increase over time (no adaptation). Regardless of the approach used, fundamental limitations in dose fractionation design and categorical pharmacodynamic classification of antimicrobial agents would still remain. However, the model does not account for the immune system (which may play an important role against small resistant populations) and bacterial stress response not observed during the initial observation period. Both issues (modeling the effect of the immune system and temporal bacterial response leading to resistance) are currently under investigation.

In summary, pharmacodynamic modeling is an important decision-support tool to guide the selection of dosing regimens. The use of surrogate pharmacodynamic indices has taught us much on the differences in the killing profiles of different antimicrobial agents, resulting in several rational dosing strategies to optimize patient outcomes. As we are dealing with drugs with more complex pharmacokinetics and pharmacodynamics, it is also clear that using simple surrogate pharmacodynamic indices may not always be informative enough to make good decisions to dosing selection. In the interest of further accelerating new drug development and for the benefits of our patients, alternative modeling and computational approaches, such as the one proposed herein should be explored.

**Author Contributions**

Conceived and designed the experiments: VHT MN. Performed the experiments: VHT MN. Analyzed the data: VHT MN. Contributed reagents/materials/analysis tools: MN. Wrote the paper: VHT.
16. Mouton JW, Vinks AA, Punt NC (1997) Pharmacokinetic-pharmacodynamic modeling of activity of ceftazidime during continuous and intermittent infusion. Antimicrob Agents Chemother 41: 733–738.

17. Tam VH, Louie A, Deziel MR, Liu W, Leary R, et al. (2005) Bacterial-population responses to drug-selective pressure: examination of garenoxacin’s effect on Pseudomonas aeruginosa. J Infect Dis 192: 420–428.

18. Tam VH, Louie A, Fritsche TR, Deziel M, Liu W, et al. (2007) Impact of drug-exposure intensity and duration of therapy on the emergence of Staphylococcus aureus resistance to a quinolone antimicrobial. J Infect Dis 195: 1018–1027.

19. Nikolaou M, Tam VH (2006) A new modeling approach to the effect of antimicrobial agents on heterogeneous microbial populations. J Math Biol 52: 154–162.

20. D’Argenio DZ, Schumitzky A (1997) ADAPT II: pharmacokinetic/pharmacodynamic systems analysis software. Los Angeles (California): University of Southern California.

21. Singh R, Ledesma KR, Chang KT, Hou JG, Prince RA, et al. (2009) Pharmacodynamics of moxifloxacin against a high inoculum of Escherichia coli in an in vitro infection model. J Antimicrob Chemother 64: 556–562.

22. Nikolaou M, Schilling AN, Vo G, Chang KT, Tam VH (2007) Modeling of microbial population responses to time-periodic concentrations of antimicrobial agents. Ann Biomed Eng 35: 1458–1470.

23. Forrest A, Nix DE, Ballow CH, Goss TF, Birmingham MC, et al. (1993) Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. Antimicrob Agents Chemother 37: 1073–1081.

24. Preston SL, Drusano GL, Berman AL, Fowler CL, Chow AT, et al. (1998) Pharmacodynamics of levofloxacin: a new paradigm for early clinical trials. JAMA 279: 125–129.

25. Louie A, Deziel M, Liu W, Drusano MF, Gumbo T, et al. (2005) Pharmacodynamics of caspofungin in a murine model of systemic candidiasis: importance of persistence of caspofungin in tissues to understanding drug activity. Antimicrob Agents Chemother 49: 5050–5068.

26. Wiederhold NP, Kontoyiannis DP, Chi J, Prince RA, Tam VH, et al. (2004) Pharmacodynamics of caspofungin in a murine model of invasive pulmonary aspergillosis: evidence of concentration-dependent activity. J Infect Dis 190: 1464–1471.

27. Bowker KE, Noel AR, Margowan AP (2008) Pharmacodynamics of minocycline against Staphylococcus aureus in an in vitro pharmacokinetic model. Antimicrob Agents Chemother 52: 4370–4373.

28. Tam VH, Schilling AN, Neshat S, Poole K, Melnick DA, et al. (2005) Optimization of meropenem minimum concentration/MIC ratio to suppress in vitro resistance of Pseudomonas aeruginosa. Antimicrob Agents Chemother 49: 4920–4927.

29. Mouton JW, Vinks AA (2005) Pharmacokinetic/pharmacodynamic modelling of antibacterials in vitro and in vivo using bacterial growth and kill kinetics: the minimum inhibitory concentration versus stationary concentration. Clin Pharmacokinet 44: 201–210.

30. Czock D, Keller F (2007) Mechanism-based pharmacokinetic-pharmacodynamic modeling of antimicrobial drug effects. J Pharmacokinet Pharmacodyn 34: 727–751.

31. Jacqumin P, McFadyen L, Wade JR (2010) Basic PK/PD principles of drug effects in circular/proliferative systems for disease modelling. J Pharmacokin Pharmacodyn 37: 157–177.