Journal of Antimicrobial Chemotherapy Advance Access published October 3, 2007

Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkm370

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Mathematical modelling response of *Pseudomonas aeruginosa* to meropenem

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Received 9 May 2007; returned 22 July 2007; revised 30 July 2007; accepted 30 August 2007

Objectives: **Widespread emergence of resistance to antimicrobial agents is a serious problem. The rate at which new agents are made available clinically is unlikely to keep up with these resistant pathogens, and there is an urgent need to accelerate antimicrobial agent development. We explored the use of mathematical modelling to guide selection of dosing regimens.**

Methods: **Using time–kill studies data of *Pseudomonas aeruginosa* over 24 h, we developed a mathematical model to capture the dynamic relationship between a heterogeneous microbial population and meropenem concentrations. The microbial behaviour in response to meropenem over 5 days was predicted via computer simulation and subsequently validated using an *in vitro* hollow fibre infection model. Three parallel differential equations were used, each characterizing the rate of change of drug concentration, microbial susceptibility and microbial burden of the surviving population over time, respectively. Several model structures were explored; they differed in the adaptation of the microbial population over time. Various fluctuating concentration–time profiles of meropenem were experimentally examined, mimicking human elimination and repeated dosing.**

Results: **Using limited experimental data as inputs, the mathematical model was reasonable in qualitatively predicting microbial response (sustained suppression or regrowth due to resistance emergence) to various pharmacokinetic profiles of meropenem.**

Conclusions: **Our results suggest that mathematical modelling may be used to predict microbial response to a large number of antimicrobial agent dosing regimens efficiently, and have the potential to be used to guide highly targeted investigation of dosing regimens in pre-clinical studies and clinical trials. The *in vivo* relevance of the modelling approach warrants further investigations.**

Keywords: pharmacodynamics, simulation, carbapenems, Gram-negative bacteria

Introduction

Resistance to antimicrobial agents is a serious problem that renders the rapid development of new agents an urgent priority. Effective treatment may not be available for common infections in the not too distant future, and we may be at risk of going back to the pre-antibiotic era in the event of an outbreak.[1] Broad-spectrum antimicrobial resistance in HIV, tuberculosis and Gram-negative bacteria (e.g. *Pseudomonas aeruginosa*, *Acinetobacter baumannii* etc.) is especially worrisome. It is therefore imperative that new and effective antimicrobial agents are developed rapidly to keep up with our combat against infections caused by these pathogens.

As widely appreciated as the magnitude of this problem may be, the current rate at which new agents are made available clinically is unlikely to meet this critical need. The traditional approach has focused on the identification of new metabolic targets and agents to interfere with essential pathways. Relatively little attention has been paid to the impact of the dosing regimen (dose and dosing frequency) of an active agent on the emergence of resistance. There are *in vitro* and *in vivo* experimental data demonstrating that dosing regimen may play an important role in the development of resistance; suboptimal dosing regimens represent a selective pressure that facilitates resistance development, whereas using optimal dosing regimens may suppress/delay the emergence of resistance.[2–4]

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However, multiple modifiable factors [e.g. the total daily dose, dosing frequency, length of (intravenous) administration and duration of therapy etc.] are involved in rational design of dosing regimens, and each factor may have a significant impact on the killing activity and propensity to suppress resistance emergence, depending on the pharmacodynamic properties of the agents and clinically achievable concentrations (associated with acceptable toxicity). The numerous combinations of these variables involved in designing dosing regimens are prohibitory for comprehensive laboratory or clinical evaluation of all the different scenarios.

Pharmacodynamic modelling has been used as a decision support tool to facilitate rational dosage design. It emphasizes the fact that effective antimicrobial treatment is attributed to neither antimicrobial agent potency (exposure) nor pathogen susceptibility alone, but rather a complex interplay of both factors. In spite of that, conventional modelling methods may be overly simplistic, relying on surrogate pharmacodynamic indices [e.g. area under the concentration–time profile (AUC)/MIC, percentage of dosing interval during which concentration is above MIC ($\%t >$ MIC) etc.] to characterize outcomes. Conventional modelling methods typically take a snapshot of microbial burden at the end of an observation period, and curve-fit the observations as a function of the surrogate index without making use of information at intermediate stages of the observation period.[5–12] Not surprisingly, these modelling approaches have restricted predictive ability and their limitations have been reviewed previously.[13] On the other hand, modelling methods that make use of all available information on microbial burden during an observation period offer distinct advantages, in terms of making useful predictions of microbial response to antimicrobial agents.[14]

We have previously developed a mathematical model to predict the response of *P. aeruginosa* to constant concentrations of meropenem.[13] In this study, we strived to enhance the clinical relevance of our modelling approach by extending the mathematical model to predict microbial response to various fluctuating drug concentration–time profiles, mimicking those resulting from different dosing regimens in humans.

Materials and methods

Antimicrobial agent

Meropenem powder was supplied by AstraZeneca (Wilmington, DE, USA). A stock solution of the drug (1024 mg/L) in sterile water was prepared, aliquoted and stored at −70°C. Prior to each susceptibility testing, an aliquot of the drug was thawed and diluted to the desired concentrations with cation-adjusted Mueller–Hinton broth (Ca-MHB) (BBL, Sparks, MD, USA).

Microorganism

P. aeruginosa ATCC 27853 (American Type Culture Collection, Rockville, MD, USA) was used in the study. The bacteria were stored at −70°C in Protect® (Key scientific products, Round Rock, TX, USA) storage vials. Fresh isolates were subcultured twice on 5% blood agar plates (Hardy Diagnostics, Santa Maria, CA, USA) for 24 h at 35°C prior to each experiment.

Susceptibility studies

Meropenem MIC and MBC were determined in Ca-MHB using a macrobroth dilution method as described by the CLSI (formerly the NCCLS).[15] The experiments were conducted in duplicate and repeated at least once on a separate day.

Time–kill studies

Time–kill studies data over 24 h have been reported previously.[13] A clinically relevant (achievable) concentration range of meropenem (0–64 mg/L) was used. A dense baseline inoculum ($\sim 2 \times 10^8$ cfu/mL) was used to simulate the bacterial load in severe nosocomial infections. These data were used as inputs to derive the best-fit model parameter estimates, as described previously.

Pharmacokinetic profiles investigated

Different pharmacokinetic profiles of meropenem were investigated for their propensity to suppress resistance. A fixed maximum concentration (C_{max}) resulting from a 1 g clinical dose (64 mg/L) with repeated dosing every 8 h (to re-attain C_{max}) was used in all the dosing regimens investigated. The dosing regimens differed in the simulated elimination half-lives (1–3 h), resulting in different concentrations at the end of the dosing interval (C_{min}). We have previously shown that high meropenem concentrations did not contribute significantly to the killing of *P. aeruginosa*.[13] From the modelling standpoint, this study design may assess the predictive performance of various mathematical models in a more robust manner.

Computer simulations

Using the best-fit model parameter values derived,[13] microbial response to various meropenem exposures over 5 days was predicted. Three parallel differential equations were used, each characterizing the rate of change of drug concentration (pharmacokinetics), microbial susceptibility and microbial burden of the surviving population over time, respectively (Figure 1). Several mathematical model structures were explored in the simulation process. These models differed in the way in which the microbial population adapted over time: (i) reversible susceptibility reduction with increasing drug concentration (selective pressure); and (ii) irreversible susceptibility reduction with increasing drug concentration. In each case, the incorporation of a biofitness cost in the bacterial growth term was also examined. The biofitness cost was not estimated from the time–kill studies, but empirically assumed to be 50% reduction in growth rate over 48 h. The performance of various mathematical model structures was assessed based on their ability to predict microbial behaviour (sustained suppression or regrowth due to resistance emergence) over time. All simulations were performed with the ADAPT II program.[16]

Experimental validation

The computer simulations were compared with experimental data from an *in vitro* hollow fibre infection model with similar antimicrobial agent exposures, as described previously.[17] Various elimination half-lives (1–3 h) of meropenem were simulated and subsequently validated in the infection models. Serial samples were obtained at baseline and daily (pre-dose) in duplicate from each hollow fibre system, for quantitative culture to define the effect of various drug exposures on the total bacterial population and on selection of resistant bacterial subpopulations. Prior to culturing the bacteria

$$\frac{\mathrm{d}C(t)}{\mathrm{d}t} = \frac{R_0}{V} - CL \cdot C(t) \quad (1)$$

$$\frac{\mathrm{d}C_{50\mathrm{keff}}(t)}{\mathrm{d}t} = C_{50\mathrm{k}} \cdot \alpha \cdot \{\mathrm{e}^{-[C(t) \cdot t \cdot \tau]}\} \cdot [C(t) \cdot \tau] \quad (2)$$

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = K_\mathrm{g} \cdot \left[1 - \frac{N(t)}{N_{\max}}\right] \cdot N(t) - \left\{ \frac{C(t)^H \cdot K_\mathrm{k}}{C(t)^H + [C_{50\mathrm{keff}}(t)]^H} \right\} \cdot N(t) \quad (3)$$

Equation (1) describes the rate of change of antimicrobial agent concentration over time.
Equation (2) describes the rate of change of bacterial susceptibility to antimicrobial agent over time.
Equation (3) describes the rate of change of bacterial population over time.

$C(t)$ — concentration of antimicrobial agent at time t
R_0 — drug infusion rate
V — volume of distribution
CL — clearance

$C_{50\mathrm{keff}}(t)$ — susceptibility of bacteria to antimicrobial agent
$C_{50\mathrm{k}}$ — concentration to achieve 50% of maximal kill rate for bacterial population
α — maximal adaptation
τ — adaptation rate function

$N(t)$ — concentration of bacterial population at time t
K_g — growth rate constant for bacterial population
$N_{\max}$ — maximum population size
K_k — maximal kill rate constant for bacterial population
H — sigmoidicity constant for bacterial population

Note 1: $C_{50\mathrm{keff}}(t) = C_{50\mathrm{k}} \cdot \{1 + \alpha[1 - \mathrm{e}^{-[C(t) \cdot t \cdot \tau]}\}$

The general solution for any $C(t)$ is:

$$\frac{\mathrm{d}C_{50\mathrm{keff}}(t)}{\mathrm{d}t} = C_{50\mathrm{k}} \cdot \alpha \cdot \{\mathrm{e}^{-[C(t) \cdot t \cdot \tau]}\} \cdot \left\{ [C(t) \cdot \tau] + \left[t \cdot \tau \frac{\mathrm{d}C(t)}{\mathrm{d}t} \right] \right\}$$

Using this simulation approach, the susceptibility of the microbial population will return to baseline in the absence of drug selective pressure (reversible).

When $C(t)$ is constant, the solution of the above equation is:

$$\frac{\mathrm{d}C_{50\mathrm{keff}}(t)}{\mathrm{d}t} = C_{50\mathrm{k}} \cdot \alpha \cdot \{\mathrm{e}^{-[C(t) \cdot t \cdot \tau]}\} \cdot [C(t) \cdot \tau]$$

Using this simulation approach, the susceptibility of the microbial population will not return to baseline in the absence of drug selective pressure (irreversible).

Note 2: $K_{\mathrm{g-eff}}(t) = K_\mathrm{g} \cdot \{0.5 \cdot [1 + \mathrm{e}^{-(0.087 \cdot t)}]\}$

$K_{\mathrm{g-eff}}(t)$ — effective growth rate of bacteria
K_g — growth rate constant for bacterial population at baseline

Using this simulation approach, the growth rate of the microbial population will decline to ~50% of its baseline value in 48 h, corresponding to the enrichment of slower growing (less biofit) but drug-resistant subpopulation in the total population.

Figure 1. Mathematical equations of the final model predicting bacterial response to various drug exposures. Patent pending.

quantitatively, the bacterial samples were centrifuged at 15 000 **g** for 15 min and reconstituted with sterile normal saline in order to minimize drug carry-over effect. Total bacterial populations were quantified by spiral plating 10× serial dilutions of the samples (50 μL) onto drug-free Mueller–Hinton agar (MHA) plates (Hardy Diagnostics). Subpopulations with reduced susceptibility (resistant) were quantified by culturing onto MHA plates supplemented with meropenem at a concentration of 3× MIC of meropenem. Since susceptibility testing is performed in 2-fold dilutions and one tube (2× in concentration) difference is commonly accepted as reasonable interday variation, quantitative cultures on drug-supplemented media plates (at 3× MIC) would allow reliable detection of bacterial subpopulations with reduced susceptibility. The media plates were incubated at 35°C for up to 24 h (total population) and 72 h

(subpopulations with reduced susceptibility), then bacterial density from each sample was estimated by CASBA-4 colony scanner/counter (Spiral Biotech, Bethesda, MD, USA). The theoretical lower limit of detection was 400 cfu/mL.

Mechanism(s) of resistance

In order to substantiate that bacterial regrowth over time was due to emergence of resistance, the susceptibility of the resistant isolates (recovered from the drug-supplemented media plates at the end of the experiments) to meropenem was repeated. The susceptibility of these meropenem-resistant isolates to a screening panel of antimicrobial agents was also performed to provide insights on the likely mechanism(s) of resistance. Based on the phenotypic profiles of the resistant isolates, the mechanism(s) of resistance was investigated using an appropriate methodology [SDS–PAGE and/or western immunoblotting (with anti-MexB antibodies)], as described previously.[17]

Results

Susceptibility studies

The MIC and MBC of meropenem for the isolate were found to be 1 and 1 mg/L, respectively.

Time–kill studies

Data from the time–kill studies and the model parameter estimates have been reported elsewhere.[13] Overall, the observed bacterial burdens over time (under constant antimicrobial agent concentrations) were reasonably described and predicted by the mathematical model.

Computer simulations and experimental validation

The pharmacokinetic simulation in the hollow fibre infection model was satisfactory (data not shown). Among the various mathematical model structures examined, the simulation approach using irreversible susceptibility reduction with increasing drug concentration was found to be the most predictive of the observed microbial behaviour. The comparisons between computer-simulated and experimental microbial responses are as shown in Figure 2. Overall, the computer predictions correlated well with experimental data qualitatively (with respect to sustained suppression or regrowth due to resistance emergence). A significant initial reduction in microbial burden was predicted for all dosing regimens examined. However, regrowth over time was predicted for suboptimal regimens with repeated dosing due to selective amplification of resistant subpopulation(s). On the other hand, sustained suppression of resistance emergence was achieved with optimal dosing regimens. Using the conventional nomenclature, the computer simulations were correct in predicting that all dosing regimens with $\%t > \mathrm{MIC}$ of $<100\%$ were associated with regrowth. In order to suppress the emergence of resistance, dosing regimens achieving $C_{\mathrm{min}}/\mathrm{MIC} \geq 4$ were necessary. Incorporating a biofitness cost to the growth term did not appear to improve the predicting ability of the mathematical models, regardless of whether susceptibility reduction over time was reversible or not (data not shown).

Mechanism(s) of resistance

The susceptibility profiles of the resistant isolates recovered are as shown in Table 1. Meropenem resistance was phenotypically stable. The mechanisms of resistance were found to be the deletion of outer membrane porin (OprD) protein and efflux pump (MexAB-OprM) overexpression, as reported previously.[17] These data provided further molecular evidence on the emergence of resistance over time, consistent with our modelling strategy to account for regrowth.

Discussion

Microbial resistance to antimicrobial agents is a rapidly spreading problem with potentially grave consequences. Many clinicians are concerned that common infections may not be treatable in the near future. Continued development of new antimicrobial agents is imperative to combat the emergence of resistance. However, traditional drug development takes many years, and it may not keep up with the emergence of resistant pathogens.

Previous studies have demonstrated the importance of the magnitude of drug exposures (resulting from different dosing regimens) in suppressing the emergence of resistance.[2–4,17] Nevertheless, information from conventional studies has not been used optimally to guide the choice of dosing regimens. Before clinical investigations in humans, various *in vitro* and animal infection models are routinely used in order to demonstrate antimicrobial activity of new compounds. Time–kill studies (based on constant concentrations of antimicrobial agents) are more economical and easier to perform; they also provide valuable information on the relationship between concentration and killing activity of the antimicrobial agent. However, hollow fibre and animal infection model studies are considered to be more clinically relevant as the concentration of antimicrobial agent fluctuates over time in these infection models, and conventional modelling approaches using surrogate indices (e.g. AUC/MIC, $\%t > \mathrm{MIC}$ etc.) could be readily employed to characterize efficacy. Furthermore, the drug exposures simulated in the hollow fibre infection model can be adjusted to mimic different pharmacokinetic profiles resulting from different dosing regimens in humans, providing insights to the efficacy of investigational agents subsequently in clinical trials. Nevertheless, in view of the multiple variables involved in dosing regimen design, comprehensive evaluation of all possible combinations is prohibitory in view of the labour-intensiveness of each investigation, even in hollow fibre or animal infection models. Often the initial choice of dosing regimens to investigate is empirically chosen (mostly trial-and-error). Lately, exploratory experiments have been performed to examine the correlation between observed killing and the most commonly used pharmacodynamic surrogate indices first, followed by dose escalation experiments to determine the optimal drug exposure.[6,9,18] Since studies with infection models are more costly and time-consuming to perform, these studies to explore the utility of various dosing regimens may not be the most efficient.

In contrast, our modelling and simulation approach offers a relatively simple method to guide testing of dosing regimens. The proposed modelling approach does not require the use of sophisticated

infection models and surrogate pharmacodynamic indices to make useful predictions of microbial response to antimicrobial exposures. Instead, standard time–kill studies data over 24 h were used as inputs to capture the relationship between drug concentrations and killing rate. In conjunction with other model parameter estimates derived (e.g. microbial growth rate, adaptation tendency) and pharmacokinetics of the antimicrobial agent, comprehensive screening of efficacy (and propensity to suppress resistance) of a large number of dosing regimens would be possible via computer simulations, but only promising agents and dosing regimens would be identified for further investigations. Under such circumstances, highly targeted pre-clinical studies with various infection models will be used to validate the computer simulations, rather than as an exploratory tool. Preliminary comparison of several related drug candidates with conflicting *in vitro* potency and pharmacokinetics (e.g. a more potent agent with a shorter elimination half-life versus a less potent agent with a longer elimination half-life) would also be possible by computer simulations.

In this study, the computer-simulated microbial response was subsequently validated using an *in vitro* hollow fibre infection model, in which the bacteria were exposed to various fluctuating meropenem concentration–time profiles. The emergence of resistance over time secondary to suboptimal meropenem exposures was ascertained by regrowth of the microbial population (after an initial decline), repeat susceptibility testing and various molecular techniques. In the pursuit of a useful mathematical model structure to predict microbial response, several model candidates with different assumptions were evaluated and the model with the most favourable predicting ability is shown in Figure 1. The modelling results revealed that susceptibility of the surviving microbial population reduced as it adapted to a drug selective pressure (i.e. becoming more resistant overall as the more susceptible subpopulations were preferentially killed), but the susceptibility did not reverse towards baseline when the drug selective pressure was no longer present. Furthermore, despite using more model parameters, predictions of microbial response to drug exposures could not be enhanced by incorporating a deficit in bacterial growth over time. Collectively, these data suggested that reduced biofitness (a metabolic compensation for acquired resistance) was not prominent when a heterogeneous *P. aeruginosa* population was exposed to meropenem.

Several studies have been published modelling similar time–kill studies data satisfactorily,[19–22] and the maximal killing rate

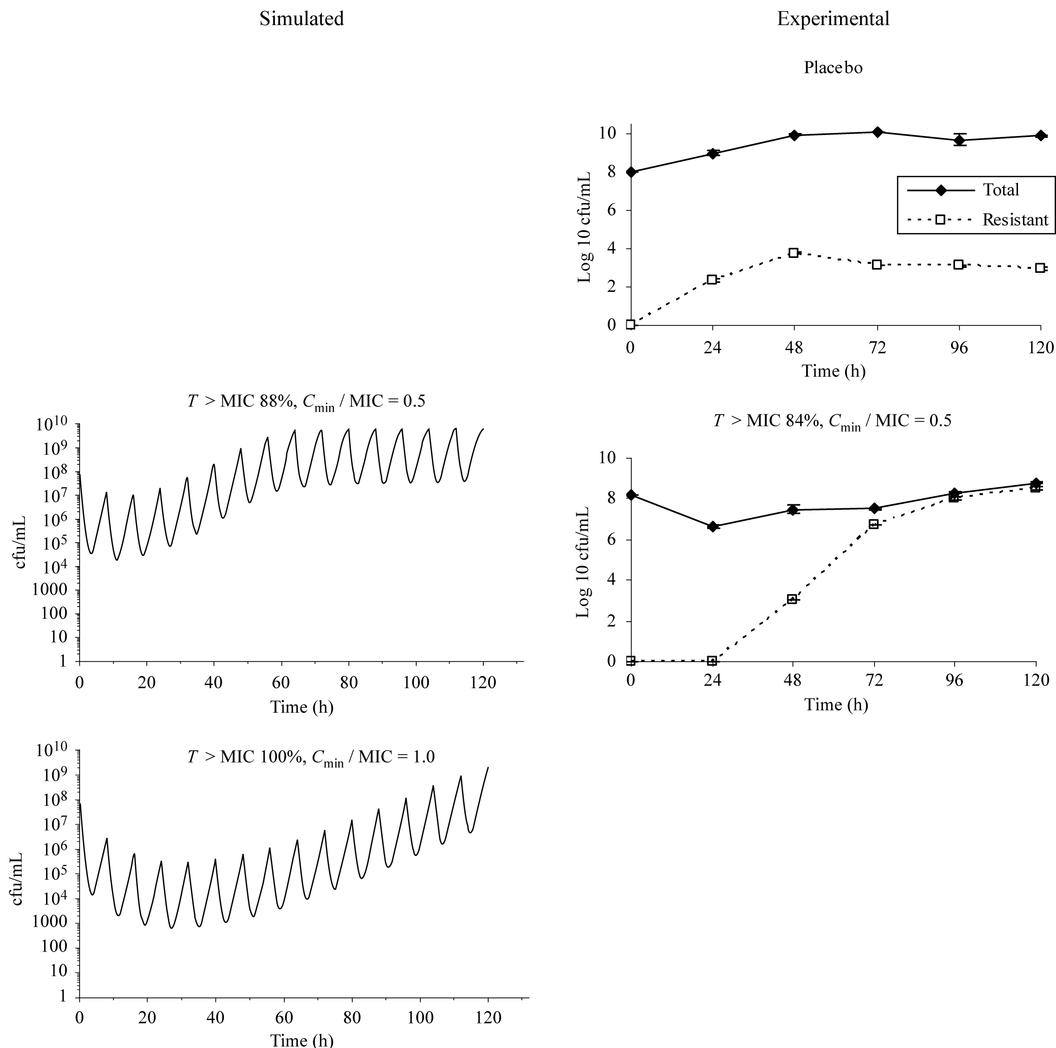

Figure 2. Comparison of computer-simulated and experimental bacterial response to various meropenem exposures. Doses were given every 8 h for 5 days in all treatment regimens. Filled diamonds, total; open squares, resistant.

Continued

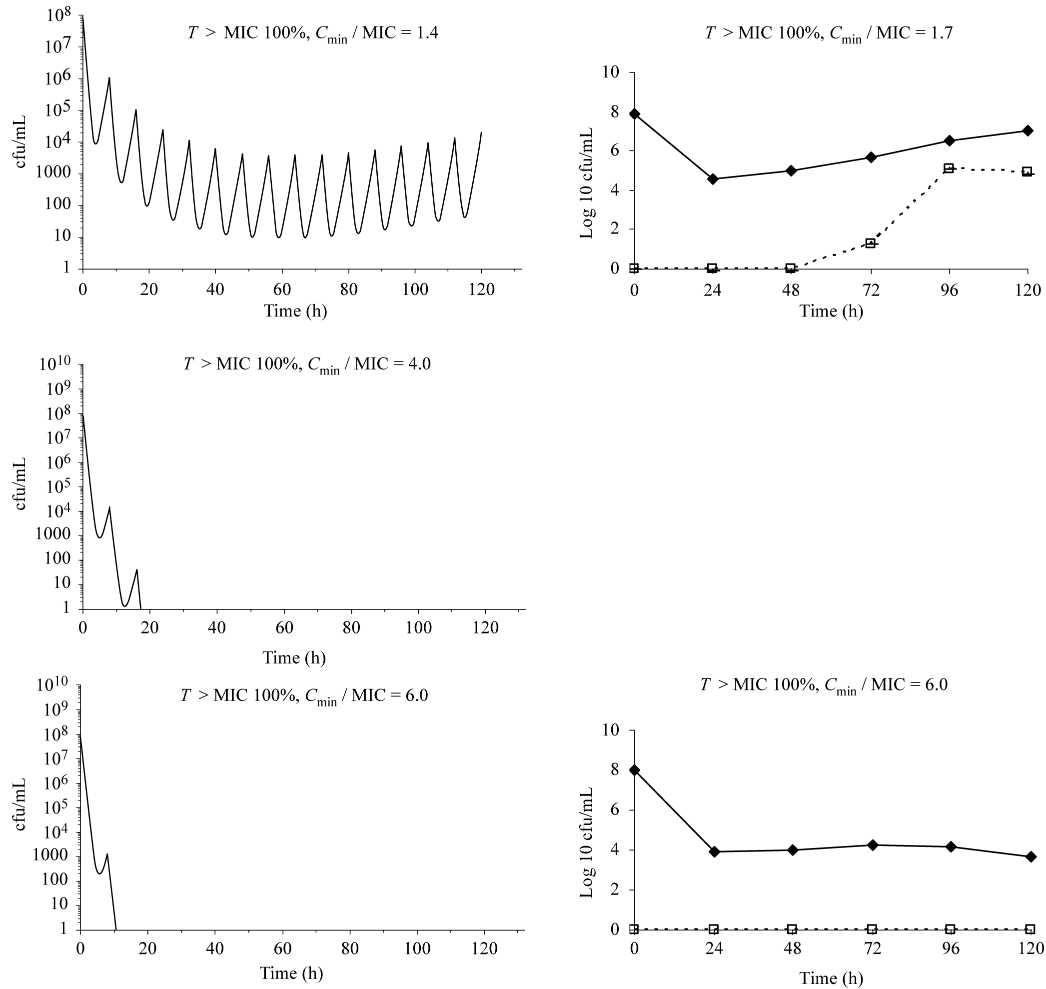

Figure 2. *Continued.*

has been modelled to be concentration/time-dependent to reflect the decrease in bacterial kill over time.[23] However, to the best of our knowledge, this is the first study in which a mathematical model could use limited (24 h) time–kill studies data as inputs to make predictions of extended (5 days) response when a microbial population is exposed to fluctuating drug concentrations. The computer predictions were validated experimentally in a qualitative nature (with respect to sustained suppression or regrowth) so far, they are nonetheless useful to guide subsequent investigations and represent a significant advancement in pharmacokinetic/pharmacodynamic modelling for antimicrobial agents. Using these computer predictions, experiments in infection models could be designed focusing on dosing regimens with a higher likelihood of success. To enhance the precision of the predictions, we anticipate further modifications in the mathematical model structure (work in progress).

Table 1. Susceptibilities of parent/resistant isolates recovered from MEM-supplemented plates at the end of experiments

Strain	Exposure (C_{min}/MIC)	MIC (mg/L)							Mechanism(s) of resistance
		MEM[a]	PIP	CAZ	IPM	LVX	TOB	CAR[a]	
PA 27853	—	1	3	1	3	0.75	0.5	64	—
MR1	placebo	4	3	1	>32	0.75	0.5	ND	OprD−
MR2	0.5	64	8	2	>32	4	0.5	512	OprD− and Mex+
MR3	1.7	32	32	4	32	6	0.5	512	OprD− and Mex+

MEM, meropenem; PIP, piperacillin; CAZ, ceftazidime; IPM, imipenem; LVX, levofloxacin; TOB, tobramycin; CAR, carbenicillin; ND, not determined; OprD−, porin deletion; Mex+, MexAB-OprM efflux pump overexpression.
[a]Meropenem and carbenicillin MICs determined by macrobroth method; other MICs determined by Etest.

Mathematical modelling and simulation hold a great potential to facilitate rational dosing design in antimicrobial agent development; they could be used to guide highly targeted testing of new agents and dosing regimens. In this study, we demonstrated a validated modelling approach using meropenem and a standard strain of *P. aeruginosa*, the choice was empiric. This approach is expected to be general and should be flexible enough to accommodate other drug–pathogen combinations. The different pharmacological (e.g. maximal kill rate, concentration dependency on killing) and microbiological (e.g. intrinsic growth rate, rate of microbial adaptation) characteristics of other combinations could be adequately captured in their respective time–kill studies and reflected in different estimates of the best-fit model parameter values (i.e. different extent of susceptibility reduction due to different mechanisms of resistance can be reflected in the value of α, and the rate of resistance selection can be reflected in the value of τ). Although theoretically feasible, the generalizability of the model would be more assured with experimental validation using more antimicrobial agents against a greater number of bacterial strains (with different mechanisms of resistance). The applicability to other drug–pathogen combinations and the *in vivo* relevance of the computer predictions are currently under investigation.

In conclusion, using limited data from time–kill studies over 24 h, the proposed mathematical model was reasonable in qualitatively predicting response of *P. aeruginosa* to various concentration–time profiles of meropenem over 5 days. The modelling approach may be incorporated in the antimicrobial agent development process to guide highly targeted investigation of dosing regimens in pre-clinical studies and clinical trials.

Acknowledgements

This study was presented in part at the Forty-fifth Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, USA, 2005 (abstract A-1826).

Funding

This study was supported in part by unrestricted grants from AstraZeneca, the Johns Hopkins Center for Alternatives to Animal Testing and a GEAR (grant to enhance and advance research) award from the University of Houston. K. P. is supported by the Canadian Cystic Fibrosis Foundation.

Transparency declarations

V. H. T. has received unrestricted research grants from AstraZeneca, Merck and Schering-Plough; he has also received speaking honoraria from Elan Pharmaceuticals. All other authors: none to declare.

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