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Pharmacokinetic/pharmacodynamic model-based combination therapy approach to target antibiotic resistant populations emerged from ciprofloxacin exposure

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Background: Despite decades of antimicrobial usage, the relationship between antimicrobial drugs and the development of drug resistance has not been fully delineated. This has led to increased frequency of resistance with increased usage of antimicrobials. In recent years, new insights into the mechanisms of antibiotic resistance have been proposed, leading to a re-evaluation of novel pharmacokinetic/pharmacodynamic (PK/PD) models. We have developed a semi-mechanistic PK/PD model to describe drug-bacteria kill curve relationships using the compensatory mutation hypothesis. In addition, we explored the model-based combination therapy approach to combat the resistance population. *Methods: In vitro* kill-curves of *E. coli 204* up to 48 h following initial ciprofloxacin (CIP) treatment at 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 125 times the minimum inhibitory concentration (MIC) totaling 193 data points were obtained with an *in vitro* system with a simulated CIP half-life of 4 h. The proposed antibiotic resistant mechanism mimics the sequential compensatory mutation hypothesis, in which mutations that acquire drug resistant traits are associated with fitness costs. Subsequent restoration of bacterial fitness is necessary for the population to be clinically relevant. Model parameters were estimated from simultaneous fitting of eleven dose groups using Adapt II software. Standard goodness of fit criteria used to obtain the final model included model convergence, Schwartz Criterion, Akaike Information Criterion, residuals versus predicted concentrations and time, and visual inspection. *Results/Conslusions:* The eleven *E. coli* kill curves after CIP treatment were well described simultaneously by the compensatory mutation model. The emergence of bacterial population with drug resistance characteristics and bacterial fitness restored appears to dominate shortly following CIP treatment. The model suggests a subsequent dose of a different mechanisms of action should be considered for the emerged resistant population.

1. Introduction

Regardless of the mechanisms of actions of antibiotic classes, microbes have been able to consistently develop resistance to new drug treatments within a few years after their implementations (Kaatz et al. 1991; Austin et al. 1999; Neuhauser et al. 2003). The major factors contributing to this predicament include lack of understanding of the underlying mechanisms of antibacterial resistance and the use of sub-optimal pressure specifically against the resistance population. The evaluation of antibiotics and microbial survival response has evolved from point estimates (minimum inhibitory concentrations) to complete time course approach (bacterial kill-curve) to reveal the drug-bacterial killing relationships (de la Pena et al. 2004; Mueller et al. 2004; Liu et al. 2005; Schmidt et al. 2008). However, it is only within recent years that the emphasis has shifted towards molecular and genetic approaches to understanding the mechanisms of resistance with additional insight on how to interpret the bacterial kill-curve relationships. It is with these new experimental findings that we propose a new mathematical model to bridge basic science research to relevant clinical usage. In this paper, we use the compensatory mutation hypothesis (Andersson and Levin 1999; Bjorkman et al. 2000; Levin et al. 2000; Martinez and Baquero 2000; Nagaev et al. 2001; Gerrish and Garcia-Lerma 2003; Gustafsson et al. 2003; Johnson et al. 2005; Rozen et al. 2007; Lofmark et al. 2008; Marcusson et al. 2009) as the foundation for the mechanism-based pharmacokinetic/pharmacodynamics (PK/PD) model. The importance of the fitness concept and genetic mutation relationship were proposed to describe antimicrobial resistance. As the microbes undergo life cycle division every 20 min, selection for drug resistance mutants occurs within a short period of time following treatment. However, mutation is often associated with a fitness cost (the rate of cell division is reduced) (Bjorkholm et al. 2001; Marcusson et al. 2009). It is only after multiple mutations that a drug resistant trait is acquired while further mutations occur and the mutated population becomes clinically relevant. It is this new compensated mutation population that explains the observed rising MIC over the time course of antimicrobial treatment (Dudley et al. 1987).

We believe a quantitative modeling approach can provide the urgently needed link between the newly proposed microbial behavior and clinical dose optimization needed to better protect against infections. In the absence of adequate new antibiotics available in the next few years, we believe our approach has great potential to revive some older antibiotics rendered useless due to resistance. The occurrence of resistance may be related to misuse of antibiotics that foster selection of resistance populations since this population was not considered when the clinical dosage was selected. In this study, we utilized a semi-mechanism-based pharmacokinetic/pharmacodynamic (PK/PD) model to explore combination therapy that considers the emerged resistance population following ciprofloxacin treatment.

2. Investigations and results

2.1. Pharmacokinetic/pharmacodynamic model descriptions

Details of the *in vitro* procedures have been published before (Firsov et al. 1997, 1998). Extensive *in vitro* kill-curve data suitable for complex mechanistic modeling were obtained using GetData Graph Digitizer 2.24 software. Briefly, a two flask system with ciprofloxacin was used against *Escherichia coli* 204 (*E. coli* II), ranging from 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 125 times the minimum inhibitory concentration (MIC) of 0.08 ug/mL. The flask containing bacteria and drug was inoculated with 18 h-cultured bacteria followed by 2 h incubation. Ciprofloxacin was injected at the 20th hour. The inoculum size at the time of treatment was approximately 10^6 colony forming units (CFU)/mL and the experiments ended when the total bacterial growth reached $\sim 10^{11}$ CFU/mL for each dose group.
A clinical half-life of 4 h for ciprofloxacin was established in an *in vitro* kill-curve system by replacing 7 mL/h of fresh media in a constant 40 mL flask. The mono-exponential decline rate was described as:

$$\frac{dC}{dt} = -kel * C \quad Initial\ Condition\ or\ IC = \frac{DOSE}{V} \quad (1)$$

where kel rate constant is fixed to 0.175 h^{-1}. A graphical description of the semi-mechanism-based PD model is shown in Fig. 1 and the respective differential equations are included below.

$$\frac{dS}{dt} = ks * S - kd * (1 + E\max, S) * S - kc * S$$

$$IC = \frac{10^6 CUF}{mL} \quad (2)$$

$$\frac{dR}{dt} = kc * S - kc * R \qquad IC = 0\frac{CFU}{mL} \quad (3)$$

$$\frac{dRfit}{dt} = ks * Rft + kc * R - kd$$

$$*(1 + E\max,\ Rfit) * Rfit \quad IC = 0\frac{CFU}{mL} \quad (4)$$

Where the stimulatory function for susceptible population is described as:

$$E\max,\ S = \left(\frac{S\max,\ S * C}{SC50,\ S + C}\right) \quad (5)$$

and for Rfit population as:

$$E\max,\ Rfit = \left(\frac{S\max,\ Rfit * C}{SC50,\ Rfit + C}\right) \quad (6)$$

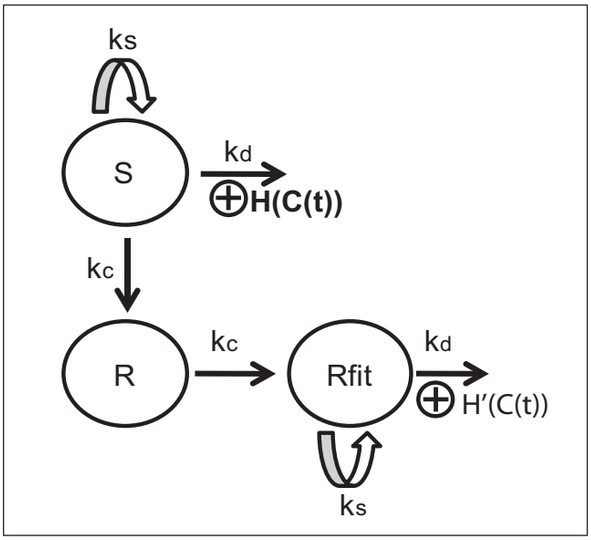

Fig. 1: Semi-mechanism-based PK/PD models for antimicrobial resistance where S = Susceptible population. Resistance population without and with fitness restored through compensatory mutations are denoted as R and Rfit. Bacterial growth and degradation rate constants are denoted as ks and kd. Mutation rate constants are denoted as kc. Independent drug effects with stimulatory Michaelis-Menten Emax model are denoted by the enclosed positive symbol

The model describes the compensatory mutation hypothesis, where S is the susceptible population, R is the mutated population with reduced fitness characteristic (negligible growth and degradation) and Rfit is the resistance population after second mutation that restores fitness while retaining drug resistance characteristics. The model assumes the fitness is fully restored, showing the Rfit population exhibiting the same growth and degradation rate constant (ks and kd) as those of the S population. The multiple mutation process is described by an arbitrary kc rate constant. Ciprofloxacin has independent nonlinear killing effect on both S and Rfit population.
A total of 10 ciprofloxacin-treated groups and one control treated group were modeled simultaneously with the PK/PD model described above using ADPT II (D'Argenio and Schumitzky, 1979). A total of 44 differential equations (sets of equations 1–4) were written to describe the eleven treatment groups. A common variant model for all 11 dose groups with maximum likelihood estimator in ADAPT II was implemented using Eq. (7).

$$var(CFU) = (\sigma 1 + \sigma 2 * Y)^2 \quad (7)$$

Where σ1 and σ2 are the variant model parameters and Y is the predicted colony forming unit (CFU) output. Standard goodness of fit criteria used to obtain the final model included model convergence, Schwartz Criterion, Akaike Information Criterion, residuals versus predicted concentrations and time, and visual inspection.

2.2. Model fitting results and diagnostics

The predicted bacterial kill-curves following ciprofloxacin treatment are shown in Fig. 2 and the associated parameter estimates are shown in the Table. The model simultaneously described the eleven dose groups reasonably well, indicating the sequential process of acquiring drug resistance mutation and restoring bacterial fitness potentially explains the mechanisms of drug resistance.
Exponential Hill factors were tested in the stimulatory function on both S and Rfit population but later removed due to values

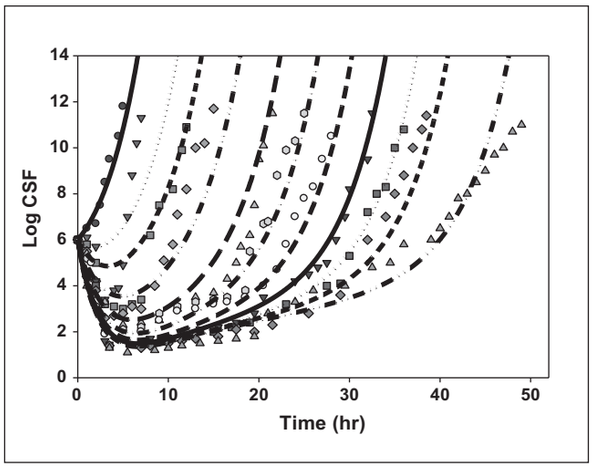

Fig. 2: Model fitting based on compensatory mutation hypothesis of *E. coli* 204 kill-curve over 48 h with ciprofloxacin. Symbols represent 10 drug treatment groups and a control treated group. From left to right, the initial ciprofloxacin concentrations were 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0 and 125 times the minimum inhibitory concentration (MIC) of 0.08 μg/mL. The respective lines represent the model predicted profiles

Table 1: Compensatory model parameter estimates

Parameter	Model Estimates	%CV
ks(/h)	0.804	14.9
Kd(/h)	0.651	18.7
Kc(/h)	0.172	10.7
$S_{MAX\ S}$	1.03	19.3
$SC_{50,S}$ μg/mL)	0.357	14.6
$S_{MAX,\ R}$	0.196	21.7
$SC_{50,R}$ μg/mL)	0.113	31.6
σ2	0.210	5.97

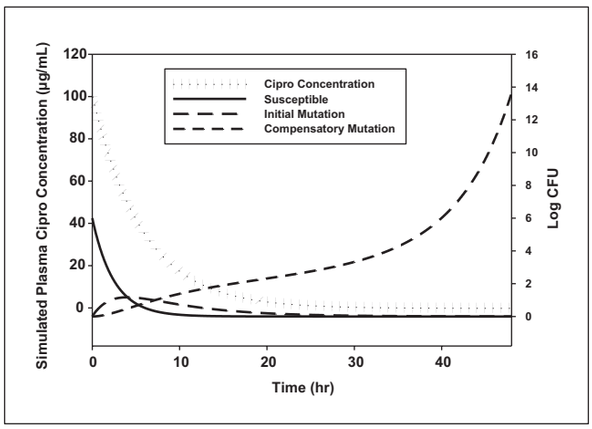

Fig. 3: The time course profiles of PK and subcompartmental PD following single ciprofloxacin treatment in *E. coli* 204. The dotted line represents the ciprofloxacin concentrations. The solid line represents susceptible bacterial population. The long dashed line describes the minimal mutated population that does not have sufficient bacterial fitness. The short dashed line that accounts for the majority of bacterial re-growth represents the drug resistant population with restored fitness

close to 1 and the absence of further objective function reductions. Variant σ1 was fixed to zero as the model estimated value was insignificant (< 0.001). To further assess the model behavior in a sub-compartmental level, the time course profile of S, R, and Rfit are shown in Fig. 3. The sub-compartmental analysis provides an insight into the temporal behavior of various proposed bacterial population that is otherwise difficult to evaluate experimentally. Figure 3 shows the drug-bacterial dynamic relationship as follows: Immediately following ciprofloxacin treatment, the susceptible population dies off in a drug concentration dependent manner. The selection pressure of drug treatment favors the emergence of a resistance population. While this population is less responsive to ciprofloxacin treatment, the cost of mutation leads to reduction in its bacterial fitness, thereby the population becomes insignificant over time. Further selection of this population with restored fitness appeared to be responsible for the re-growth of the bacterial population. As shown in the Table 1, the Rfit population has a greatly reduced overall response to ciprofloxacin as compared to the susceptible population (Smax, R versus Smax, S).

2.3. Exploratory mechanism-based combination therapy

Antibiotic resistance is associated with increased MIC following drug treatment. Although MIC distribution over time was not assessed in the current study, a reduction in drug effects indirectly infers a rise of MIC during the drug exposure period. Using the time course relationship of sub-compartmental analysis from Section 2.2, additional simulations were explored to assess the feasibility of combination therapy. Figure 4 shows the proposed combination therapy approach using the semi-mechanism-based PK/PD model. It simulates scenarios where a second drug is inhibiting the production of bacterial synthesis with IC50 set at 0.1, 1, and 10 μg/mL using Eq. (8).

$$K\max = \left(1 - \frac{I\max * C}{IC50 + C}\right) \tag{8}$$

where Kmax is the inhibitory equation on the synthesis rate constant of Rfit population. Imax is the maximum inhibitory effect, which was fixed to the maximum of 1 in the simulation. IC50 represents the drug potency.

Provided the reduction of the ciprofloxacin response after the initial dose, an antibiotic drug from a different class that exhibits an alternative pharmacological action given as the second dose has greater beneficial effects compared to continuing ciprofloxacin mono-therapy at the same or elevated (3X) dose.

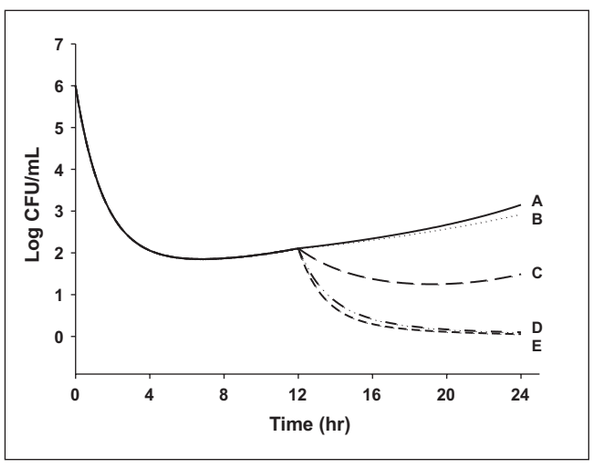

Fig. 4: A twice a day dosing simulation of 200 mg ciprofloxacin on the first dose, and 200 mg ciprofloxacin on the second dose (A), or 800 mg the second dose (B). An alternative drug inhibiting bacterial growth of Rfit rather than stimulating bacterial killing with IC50 of 10 μg/mL (C), 1 μg/mL (D), and 0.1 μg/mL (E) administered as the second dose may be more effective than continuation of ciprofloxacin treatment, even if the ciprofloxacin dose is increased three fold

3. Discussion

The semi-mechanism-based PK/PD model mimicking the compensatory antimicrobial resistance hypothesis appears to describe the effects of eleven ciprofloxacin dose groups simultaneously reasonably well. The model captures the multiple step process needed for bacteria to develop into a clinically relevant resistant population. The resistance without fitness compartment (R) explains the findings that mutated bacteria often lead to lower bacterial growth. Subsequent mutations that retain the drug resistance characteristics while acquiring the restoration of fitness from the clinically resistant populations that should be the target of interest.

In theory, the second clinical dose would render diminished drug response with increased MIC. This phenomenon was observed by Dudley and his colleagues using a multiple dose *in vitro* kill-curve system of ciprofloxacin against *P. aeruginosa* (Dudley et al. 1987). Their findings show that bacterial killing at the second dose (12 h) did not show an apparent reduction as compared to the first dose. In addition, the profiles of MIC distributions at 12 h were shown to increase by a large degree compared to the initial drug exposure. This multiple dose profile appears to be explainable by our compensatory PK/PD model. The multiple dose simulation from the model also did not show an apparent bacterial killing profile at a second dose given at 12 h compared to the initial drug exposure. In our simulation, a three-fold increase in ciprofloxacin dosing only slightly increased bacterial killing (Fig. 4). This lead us to explore novel dosing schemes using bacterial behavior information extracted from the PK/PD model.

In general, a higher initial dose may result in a more pronounced pharmacodynamic effect, assuming maximum effects have not been reached. However, higher doses increase the risks of toxicity to patients. Hence, an alternative class of antibiotics with a different mode of pharmacological action may be more effective against the merged resistant population. At various potency levels (IC50), the model suggests a second drug with different pharmacological actions given at a subsequent dose may be more beneficial than continuation of ciprofloxacin where the bacterial have already evolved to successfully resist and grow with comparable fitness as that of the parent population.

In conclusion, this paper demonstrates a novel semi-mechanism-based PK/PD model that describes the complex mechanisms of antimicrobial resistance using findings from recent basic science research. The microbial behavior extracted from the developed model was used to propose a study design for future experiments, predicting a different class of drug at a subsequent dose may result in superior killing of the resistant population compared to a continued treatment with ciprofloxacin.

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