Review Article

Making the Most of Clinical Data: Reviewing the Role of Pharmacokinetic-Pharmacodynamic Models of Anti-malarial Drugs

Julie A. Simpson,^{1,5} Sophie Zaloumis,¹ Alysha M. DeLivera,¹ Ric N. Price,^{2,3} and James M McCaw^{1,4}

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Abstract. Mechanistic within-host models integrating blood anti-malarial drug concentrations with the parasite-time profile provide a valuable decision tool for determining dosing regimens for anti-malarial treatments, as well as a formative component of population-level drug resistance models. We reviewed published anti-malarial pharmacokinetic-pharmacodynamic models to identify the challenges for these complex models where parameter estimation from clinical field data is limited. The inclusion of key pharmacodynamic processes in the mechanistic structure adopted varies considerably. These include the life cycle of the parasite within the red blood cell, the action of the anti-malarial on a specific stage of the life cycle, and the reduction in parasite growth associated with immunity. With regard to estimation of the pharmacodynamic parameters, the majority of studies simply compared descriptive summaries of the simulated outputs to published observations of host and parasite responses from clinical studies. Few studies formally estimated the pharmacodynamic parameters within a rigorous statistical framework using observed individual patient data. We recommend three steps in the development and evaluation of these models. Firstly, exploration through simulation to assess how the different parameters influence the parasite dynamics. Secondly, application of a simulation-estimation approach to determine whether the model parameters can be estimated with reasonable precision based on sampling designs that mimic clinical efficacy studies. Thirdly, fitting the mechanistic model to the clinical data within a Bayesian framework. We propose that authors present the model both schematically and in equation form and give a detailed description of each parameter, including a biological interpretation of the parameter estimates.

KEY WORDS: anti-malarial treatment; Bayesian methods; parameter estimation; pharmacokinetic-pharmacodynamic model; *Plasmodium falciparum*.

INTRODUCTION

Malaria remains one of the most important parasitic diseases of humans. Globally each year, about a quarter of a billion people become ill with clinical malaria, of whom approximately half a million die (1). Over the last decade, huge progress has been made in improving malaria control programs and reducing the burden of disease. A key element of successful control programs is ensuring rapid diagnosis and highly effective anti-malarial treatment. The WHO currently recommends artemisinin-based combination therapies

(ACTs) as the first line treatment of falciparum malaria (2), to improve anti-malarial efficacy, reduce malaria transmission, and minimize the selection of drug-resistant parasites (3). In this context, the optimal deployment of anti-malarial treatment regimens is imperative to ensure rapid and reliable cure of the patient and prolong the useful life of drugs against emerging multidrug resistance.

Mechanistic pharmacokinetic-pharmacodynamic models (PK-PD) provide a useful means of studying drug action, whereby, drug treatment outcomes can be simulated for hypothetical individuals (4). Mechanistic PK-PD models for malaria characterize the relationship between the anti-malarial drug concentrations in the blood (the pharmacokinetic (PK) data) and the parasite-time profile (pharmacodynamic (PD) response) within an individual host. Such quantitative models can aid decision making regarding the choice of dosing schemes for current ACTs and new anti-malarials, which can then be selected for evaluation in clinical studies (5–7). These models can be incorporated in population-level analyses to predict the selection and spread of drug resistance (8). A variety of within-host PK-PD models have been described for falciparum malaria, and there is a wide variation in the number of biological parameters describing

¹Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia.

²Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, UK.

³Global Health and Tropical Medicine Division, Menzies School of Health Research, Charles Darwin University, Darwin, NT, Australia.

⁴Murdoch Childrens Research Institute, The Royal Children's Hospital, Parkville, Victoria, Australia.

⁵To whom correspondence should be addressed. (e-mail: julieas@unimelb.edu.au)

the parasite life cycle and PD effect of the anti-malarial treatment (5–7,9–22). The accuracy of these models in predicting the change in parasite burden following treatment depends on many factors. The models need to capture comprehensively the mechanisms (known or hypothesized) of parasite replication and the killing action of the drug. It is critical that the within-host PK-PD model has adequate structure to represent the key underlying biological processes. The validity of simulated parasite-time profiles relies on the accuracy of the input values of the model.

Some of these mechanistic PK-PD models have been fitted formally to patient parasite-time profiles to obtain parameter estimates (10,11,14,16,17,21,22), whereas others have simply selected parameter values that generate parasite-time profiles that accord well with the profiles from clinical studies (5–7,9,12,13,15,18–20). Both of these approaches are dependent on empirical data defining the dynamics of the parasite burden. The latter is generally derived by microscopic examination of the blood of patients with malaria, with its accuracy varying considerably according to laboratory practice and the expertise of the technician.

The development and evaluation of reliable within-host PK-PD models are becoming increasingly relied upon to optimize anti-malarial treatment regimens to maximize the impact of policy and elimination efforts and contain the emergence of anti-malarial resistance (23–26). In this review, we describe the within-host PK-PD models for anti-malarial drugs that have been developed over the last two decades and discuss the challenges of applying them to clinical data within a formal statistical framework. We conclude with recommendations for the evaluation of these models prior to fitting the model to clinical data, deriving the model parameters and interpreting results.

WITHIN-HOST ANTI-MALARIAL PHARMACOKINETIC-PHARMACODYNAMIC MODELS

Figure 1 provides a schematic illustration of the steps involved in the development and evaluation of PK-PD models for anti-malarial therapies. In this section, we review the mechanistic PK-PD models that have been developed to describe the relationship between the effects of the anti-malarial drug(s) on parasite burden over time and how the models capture the biology of malaria infection (see Fig. 1a–d).

The *Plasmodium falciparum* parasite burden changes over time in the presence of anti-malarial treatments. This process results from an interchange between parasite growth (due to asexual reproduction of the parasite every 48 h, see Fig. 1d) and the rate at which the parasites are killed by the drug and the host's immune response.

Early literature quantified this relationship with a single ordinary differential equation (ODE; see Eq. 1 below) (13,15,18) :

$$\frac{dP}{dt} = P(t) \times (k_{\text{PMF}} - k_{\text{drug}}(t)) \quad (1)$$

where $P(t)$ represents total parasite burden at time t , k_{PMF} is the parasite-growth-rate constant to account for the asexual reproduction at the end of the life cycle (PMF

denotes parasite multiplication factor), and k_{drug} is the killing rate constant of the drug (see Fig. 2a). In this model, k_{PMF} was assumed to stay constant throughout the duration of the infection, whereas k_{drug} depends on the drug concentration level and time. Figure 1a provides a simulated example of a drug concentration *versus* time profile (a PK model with first-order absorption and elimination). Figure 1b illustrates the relationship k_{drug} *versus* drug concentration profile (a nonlinear relationship is presented where the effect of the drug saturates in a sigmoidal fashion). Figure 1c shows the resulting relationship between k_{drug} and time. Recently, this single ODE model has been employed to evaluate the effectiveness of the ACTs, in particular, as artemisinin resistance spreads (5,7,20).

These simple within-host models ignored limitations on parasite growth due to both host resource (erythrocyte) availability and immunity (both innate and acquired) and the stage-specific action of the anti-malarial treatments. In other words, parasite-time profiles simulated from the model presented above (Eq. 1) apply only to a nonimmune individual administered an anti-malarial drug that is active against all stages of the parasite life cycle, and the results may only be valid for the initial posttreatment follow-up (~1–2 weeks), since modeling of historical data from syphilis patients receiving malaria therapy observed acquired immunity to take effect about 8 days (range, 4 to 14) postmicroscopic detection (27). Whether or not reductions in resource availability play a substantial role in parasite growth over time remains an open and difficult to address question which we return to in the “DISCUSSION.” Austin *et al.* published (9) an extended version of the above within-host model, which included compartments for immunity and uninfected red blood cells as well as the above single compartment for erythrocytes infected with parasites, and more recently, Winter and Hastings (5) presented for illustrative purposes only an empirical function for immunity in the supplemental material that could be incorporated into future PK-PD models.

To incorporate more detail of the biology of the intra-erythrocytic life cycle of *P. falciparum*, the above single ODE (Eq. 1) was extended to have separate ODEs that describe the movement of the parasites over time across multiple parasite compartments that represent the different stages of the *P. falciparum* life cycle (see Fig. 1d for the parasite life cycle within the red blood cell). These more complex models, described in the succeeding paragraphs, allow simulation of the total and within each stage parasite load, as opposed to simply the total parasite burden. The extended models also facilitate incorporation of the parasite stage-specific killing action of the anti-malarial treatments. For example, the artemisinin derivatives are active against rings and trophozoites (28) whereas mefloquine is only active against trophozoites (29).

For severe falciparum malaria, a model was developed with two compartments representing the parasite burden $P_1(t)$, for circulating parasites (aged 1–24 h) and the parasite burden $P_2(t)$, for sequestering parasites (aged 25–48 h, these older parasites cause the red blood cell to stick to the wall of the blood vessel) at time t (11). This model included five parameters: two transition rate constants for movement from circulating to sequestering compartment (k_{12}) and *vice versa* (k_{21}) (the inverse of these transition rates is the average time

Fig. 1. Schematic of steps involved in the development, evaluation, and utilization of anti-malarial pharmacokinetic-pharmacodynamic (PK-PD) models: Step I: Modeling the relationship between drug concentration and time (a), killing rate constant of drug (k_{drug}) and drug concentration (b), and thus, the relationship between k_{drug} and time (c). Step II: Incorporating the biology of the intra-erythrocytic life cycle of *P. falciparum* (d). Step III: Estimation of the parameters and model validation using clinical data (e observed parasitaemia (PD measure) versus time data from clinical studies). Step IV: Simulation of circulating and sequestered parasite burden over time profiles following anti-malarial therapy to investigate different dosing regimens (f simulated drug concentration versus time profiles (red line dihydroartemisinin, purple line mefloquine, based on values in the literature derived from population PK modeling) and circulating (black line) and sequestered (blue line) parasitaemia versus time profiles (simulated using discrete-time stage-specific within-host PK-PD model) (6))

spent in that compartment), two killing rate constants (k_{death1} and k_{death2}) representing the rate at which the parasites are removed from the circulating and sequestering parasite compartments respectively, and the PMF (for replication and invasion of red blood cells). Refer to Fig. 2b for a visual representation. This mechanistic PD model assumed that the killing rate constants did not change over time, in other words, these killing rate constants were not linked to the drug concentration time profile of the patient (*i.e.*, the PK profile). Furthermore, there was no parameter(s) included to represent immunity or resource limitations of the erythrocyte population. Instead, the authors stated that the killing rate constants encompassed both host immunity and the effects of the anti-malarial therapies. The authors, in another publication (22), also expanded the above model to have 48 parasite compartments of an average age of 1 h ($P_1(t)$, $P_2(t)$, ... $P_{48}(t)$). The model comprised of four parameters: a single transition rate for moving between the 48 compartments and two killing rate constants for different groupings of the parasite compartments (selected

based on *in vitro* data regarding the killing action of the anti-malarial) and PMF.

The above age-structured continuous-time model was extended to a model which represented parasite burden at time t in four compartments ($P_1(t)$, $P_2(t)$, $P_3(t)$, and $P_4(t)$), each representing 12 h of the 48-h life cycle, and in a further fifth compartment ($P_5(t)$) for removal of the damaged circulating parasites by the spleen (17). As for the previous models, no host immune response or resource limitations of the erythrocyte population were included as separate parameters. The elimination of the parasites from the parasite compartments to the "spleen" compartment was assumed to be due to the effect of the anti-malarial treatments, k_{drug} . For mefloquine (long-acting anti-malarial treatment with elimination half-life of 1–2 weeks (30)), this kill rate constant was assumed to depend on the drug concentration in a linear relationship. For artemisinin (elimination half-life of 2 h (31)), k_{drug} was modeled as a near-instantaneous removal of a large proportion of circulating parasites to the spleen at the time of drug administration (Eq. 6 (17), where one must note

Fig. 2. Continuous-time within-host pharmacokinetic-pharmacodynamic models: **a** Total parasite burden models (5,7,13,15,18,19,20); **b** Stage-specific models (11). Note, k_{death} was assumed to be determined by the killing action of the anti-malarial and host immunity; **c** Stage-specific models (10,17,21). Note: Gordi *et al.* had only three compartments for the parasite life cycle and the drug only had action ($k_{drug}(t)$) against two of these compartments

that a “slope parameter” γ was set to 45, an extreme value), although the interpretation of the formula given by the authors suggests a somewhat different intention. Furthermore, in the case of a reasonable slope parameter, the formula results in k_{drug} decreasing in value at a time when the drug concentration is increasing. The model is summarized in Fig. 2c, where k_{pq} denotes the transition rate constant for movement from compartment p to q . Variations of this five-compartment model have also been published (10,21), and of note, both papers model the relationship between artemisinin (10) (and artemether/dihydroartemisinin (21)) concentration and killing differently to (17), thereby avoiding the issue just discussed.

For the age-structured continuous time models, the distribution of the number of parasites within each compartment at the onset of treatment was derived differently for the models. Gravenor *et al.* and Gordi *et al.* assigned the number of parasites in the first compartment (P_1) to be equal to the measured baseline parasitaemia by microscope, and the number of parasites in compartment 2 were estimated from the data (note, Gordi *et al.* also had parasite compartments 3 and 4; these were assumed to have zero parasites at time of treatment) (10,11). Svensson *et al.* introduced a base number of parasites into compartment 1 (*i.e.*, P_1) 96 h before treatment (*i.e.*, two life cycles), thus parasites were distributed across all compartments at onset of treatment (17). An additional lag time parameter was included to allow patients to be infected at different times prior to treatment. Hietala *et al.* introduced parasites into P_1 four life cycles prior to treatment (21).

A consequence of modeling the parasite life cycle (*i.e.*, the ageing process of parasites) as a system of ODEs (continuous-time PK-PD models) with first-order coupling between states is that waiting times in each stage of the parasite life cycle are exponentially distributed. That is, while the expected (mean) time spent by parasites in a given age interval is correct (*e.g.*, 12 h in the 1- to 12-h class), any individual parasite may be considered to have its life-time in that compartment exponentially distributed, with the result that the modeled ageing process does not accurately reflect biological reality.

Paralleling approaches to modeling infectious diseases transmission in age-structured populations in epidemiology, others have proposed discrete-time models to incorporate the age of the parasite (12,14,16). Adopting a discrete-time framework provides all parasites to transition through age classes at precisely the expected (mean) age, providing arguably improved biological fidelity. For these models, the life cycle is divided into intervals and expressed by the following difference equations:

$$P_{i,a}(t) = P_{i,a-1}(t-1) \times S_{a-1}(t-1) \quad \text{for } a = 2, 3, \dots, n_a \quad (2)$$

$$P_{i,1}(t) = PMF \times P_{i,n_a}(t-1) \times S_{n_a}(t-1) \quad \text{for } a = 1 \quad (3)$$

where $P_{i,a}(t)$ represents the expected number of parasites aged a for patient i at time t and $P_{i,a}(t)$ depends on the number at the previous time point and age interval, as well as

the stage-specific killing effect of drug (defined as function $S_a(t)$ above). As described previously, PMF represents the number of parasites after asexual reproduction at the end of the 48-h cycle that successfully invade the red blood cells. See Fig. 3 for a pictorial representation of the above parasite-age-structured discrete-time model.

Saralamba *et al.* and Hoshen *et al.* divided the parasite life cycle into hourly intervals (*i.e.*, n_a equals 48) (12,14) whereas Smith *et al.* used 12 intervals of equal four hourly duration (*i.e.*, n_a equals 12) (16). To allocate the number of parasites at time zero (*i.e.*, prior to treatment) to each of the age intervals, a , Hoshen *et al.* (12) assumed a uniform distribution, Saralamba *et al.* (14) assumed parasite age was normally distributed (sampling range for simulation was 4 to 16 h for mean age of parasites), and Smith *et al.* (16) used a circular function based on the von Mises distribution (32), which allows the patient to have a synchronized component and an unsynchronized component across the 12 four hourly stages of the parasite life cycle.

$S_a(t)$ in Eqs. 2 and 3 represents the proportion of parasites aged a that survive time interval t and is a function of drug concentration and time. All of the published discrete-time models assume the relationship between drug effect and concentration to be sigmoidal (see Fig. 1b), as observed for *in vitro* data. Furthermore, all three models did not include host immunity (and resource availability of the red blood cells) and thus assumed that all parasite killing was due to exposure to the anti-malarial treatments. This will only prove a limitation if the model is used to consider parasite kinetics beyond 2 weeks, when it is understood that host resource limitations and/or immune responses would naturally limit continuing exponential parasite growth (27). Of note, Hoshen *et al.* (12) expanded the model to allow for parasitized red blood cells exposed to the artemisinin derivatives to become dormant and die at a later time.

PARAMETER ESTIMATION FROM CLINICAL DATA

The ultimate aim of mechanistic anti-malarial PK-PD models is to predict the parasite response to anti-malarial treatment accurately in patients infected with malaria (see Fig. 1f for simulated circulating and sequestered parasite count *versus* time profiles for a patient receiving artesunate plus mefloquine). The success of these models depends heavily on the available clinical data from which parameters can be estimated and the model validated. The parasite count determined by the microscope may not accurately reflect the true parasite burden. Furthermore, the number and timing of blood samples collected for determining both blood drug concentrations and parasite burden are limited due to ethical and logistical constraints (33,34). PK-PD models are highly nonlinear with many parameters, and this introduces statistical challenges with regard to structural and/or deterministic identifiability of all the parameters.

Summary and Limitations of Available Clinical Data

The primary PD measure for anti-malarial efficacy is the asexual parasite density within the peripheral blood circulation of an infected patient. This is usually assessed prior to initial treatment, to confirm the diagnosis and the species of infection. The parasite density reflects delays in a patient seeking treatment, as well as host immunity which contributes to controlling within host parasite growth. The parasite biomass is critical in determining the speed of parasite clearance, the risk of developing severe manifestations of disease, and the risk of subsequent recrudescence infections (the reappearance of parasites from the same initial infection above the limit of microscopic detection) (35). Other clinical and parasitological outcomes include the prevalence of gametocyte carriage which underlies the transmission

Fig. 3. Discrete-time within-host pharmacokinetic-pharmacodynamic model where $P_{i,a}(t)$ is the number of parasites aged a for individual i at time t where $a=1, 2, \dots, n_a$. $P_{i,a}(t)$ depends on the number at the previous time point and age interval, as well as $S_a(t)$, the stage-specific killing effect of the anti-malarial. Parasite multiplication factor (PMF) represents the number of parasites after asexual reproduction at the end of the 48-h cycle that successfully invade the red blood cells

potential of the parasite to the mosquito vector and the hematological recovery following malaria (36,37).

In clinical trials of uncomplicated malaria, peripheral blood films are examined by microscopy, which is usually repeated once daily until the patient's parasite density falls below the limit of detection. Subsequent follow-up is usually undertaken weekly for the duration of follow-up (at least 28 days) to detect evidence of recurrent parasitaemia. In clinical trials of severe malaria, patients are more unwell and parasite density is often assessed more frequently, up to four or six hourly. The parasite density determined by microscopy represents the number of circulating parasites per unit volume of blood (see Fig. 1e for observed circulating parasite density *versus* time profiles in the first 24 h following treatment). Parasites in the latter half of the life cycle have a propensity to adhere to the endovascular lining of blood vessels, and hence are usually not visible in the peripheral blood film and thus excluded in the quantification of whole body parasite biomass. This biomass may represent up to 50% of the total parasite load (11) and thus a major confounder in the application of these mechanistic PK-PD models to parasitaemia determined by microscopy. For simple ODE models, whole body parasite biomass is predicted by the model (Fig. 2a). For the age-structured models the sum of the parasites in the compartments visible by microscopy are fitted to the observed circulating parasite densities (e.g., P_1 in Fig. 2b). Those models which included a spleen compartment (10,17,21), assume that the parasites within this compartment are quantifiable by microscopy. Quantitative assay measurements of the Histidine-rich Protein II enzyme can be used to calculate whole parasite biomass (38,39) but these assays are not routinely used to measure parasite clearance due to expense and logistical constraints.

The limit of detection of parasitaemia by microscopy varies from 10 to 50 parasites per microliter of blood, depending on the expertise of the microscopists and the number of fields examined; this range corresponds to a whole of body parasite burden of 5×10^7 to 2.5×10^8 parasites for an adult. Most patients treated with an ACT clear their detectable peripheral parasitaemia within 48 h, although parasites can remain undetected in the bloodstream for 1–2 weeks or longer. More sensitive PCR methods (40,41) have been developed to detect lower parasite burdens, the current limit of detection being approximately 2×10^3 parasites, but these methods are not widely available.

Clinical trials provide important *in vivo* observations of anti-malarial activity but are confounded by a variety of host, drug and parasite factors, as well as the study design. Complementary data on the parasite drug response can be provided by *in vitro* drug susceptibility testing which assesses the inhibition of parasite growth following exposure to different concentrations of the anti-malarial drugs (42). Data from *in vitro* experiments facilitates monitoring for drug resistance (43), provides information regarding the relative PD of different anti-malarial agents (28,44) and the effect of the anti-malarial on different stages of the parasite life cycle (28). The usual parameter reported is the EC_{50} or drug concentration resulting in 50% inhibition of the maximal drug effect. However the absolute *in vitro* EC_{50} drug concentration

for each anti-malarial does not accord well with observed *in vivo* predictions (6).

Statistical Approaches for Estimation of the Biological Parameters

As is the case in other host-pathogen systems (e.g., influenza viral dynamics (45)), and biology in general (46), there is an inherent tension in model specification in balancing the need for biological fidelity with the ability to estimate model parameters from available data. Furthermore, for complex and highly parameterized models, determining whether or not a model is structurally identifiable (*i.e.*, if given the available data, model parameters are able, even in principle, to be uniquely determined) is challenging (47).

Reflecting these tensions, and as outlined earlier and summarized in Table I, existing PK-PD models of malaria infection dynamics involve differing levels of biological complexity which require a range of approaches to parameter specification or estimation. A number of studies have not attempted formal parameter estimation and simply draw on the existing literature to specify model parameters that are both biologically plausible and result in observed parasite kinetics that mimic experimental observations (5–7,9,12,13,15,18–20) (Table I). Whilst potentially providing useful therapeutic insights, it is possible that an alternative model parameterization may exist that could represent the observed data equally well but provide markedly different conclusions in terms of the magnitude of the killing effect of the drug and the shape of the concentration-effect relationship. Others have used statistical estimation approaches (e.g., maximum likelihood methods) that will often converge at a set of parameter estimates that do not provide the optimal fit of the model to the data, and as above, alternative parameterizations may exist. Relatively few anti-malarial PK-PD models have employed more rigorous statistical fitting procedures that explore the entire joint distribution of all parameters to find a global maximum.

Saralamba *et al.* (14) implemented a Monte Carlo optimization approach, simulating 100,000 sets of parameters and selecting out those which minimized the residual sum of squares. This technique was applied separately to each individual parasitaemia *versus* time curve (drawn from 79 patients enrolled in a clinical efficacy study), instead of simultaneously modeling all patient data and including the hierarchical nature of the data in the statistical model. In PK modeling, this simple two-stage approach (*i.e.*, estimating from each individual profile separately and combining the estimates to derive the population distribution) has been evaluated in simulated studies and shown to inflate the between-individual variability and potentially produce biased estimates (48).

Nonlinear mixed-effects (NLME) modeling is an alternative and more parsimonious modeling framework, which respects the hierarchical nature of parasitological and clinical data. NLME involves a single model and fitting step (unlike the separate fit approach). Svensson, Gordi and Hietala *et al.* (10,17,21), investigating the PD response of different anti-malarial therapies (see Table I), employing NLME models with maximum likelihood estimation. Likely reflecting

Table 1. Summary of Existing Pharmacokinetic-Pharmacodynamic (PK-PD) Models of Falciparum Malaria Infection

Paper	Primary purpose	Statistical approach for estimation of pharmacodynamic parameters
Continuous-time PK-PD models		
Single compartment for parasite burden in red blood cell		
Austin <i>et al.</i> (9)	Development of PK-PD model (additional compartments for uninfected red blood cells and free merozoites)	No estimation of parameters from clinical data Simulated PK and PD curves based on chosen values of PK-PD parameters from other literature. Deterministic simulations performed
Hoshen <i>et al.</i> (18)	Development of PK-PD model for chloroquine monotherapy	No estimation of parameters from clinical data Simulated PK and PD curves based on chosen values of PK-PD parameters from other literature. Deterministic simulations performed Simulated PD curves compared with summaries of observed parasitaemia <i>versus</i> time curves from published clinical studies
Simpson <i>et al.</i> (15)	Development of PK-PD model for mefloquine monotherapy	No estimation of parameters from clinical data Simulated PK and PD curves based on chosen values of PK-PD parameters from other literature and also from nonlinear mixed-effects modeling of <i>in vitro</i> data (maximum likelihood estimation). Deterministic simulations performed
Hoshen <i>et al.</i> (13)	Development of PK-PD model for mefloquine monotherapy	No estimation of parameters from clinical data Simulated PK and PD curves based on chosen values of PK-PD parameters from other literature. Deterministic simulations performed Simulated PD curves compared with summaries of observed parasitaemia <i>versus</i> time curves from published clinical studies
Hoshen <i>et al.</i> (19)	Extension of PK-PD model for combination therapy—artesunate and mefloquine (absorption phase of drug concentration-profile ignored, assumed at maximum concentration at time 0)	No estimation of parameters from clinical data Simulated PK and PD curves based on chosen values of PK-PD parameters from other literature. Deterministic simulations performed for PD parameters and stochastic simulations ($n=20$) for PK parameters Simulated PD curves compared with summaries of observed parasitaemia <i>versus</i> time curves from published clinical studies
Winter and Hastings (5)	Extension of PK-PD model for artemisinin-based combination therapies (absorption phase of drug concentration-profile ignored, assumed at maximum concentration at time 0)	No estimation of parameters from clinical data Simulated PK and PD curves based on chosen values of PK-PD parameters from other literature. Stochastic simulations ($n=10,000$) performed Simulated PD curves compared with summaries of observed parasitaemia <i>versus</i> time curves from published clinical studies
Kay and Hastings (7)	Extension of PK-PD model for artemisinin-based combination therapies (absorption phase of drug concentration-profile included)	No estimation of parameters from clinical data Simulated PK and PD curves based on chosen values of PK-PD parameters from other literature. Stochastic simulations ($n=10,000$) performed Simulated PD curves compared with summaries of observed parasitaemia <i>versus</i> time curves from published clinical studies
Hodel <i>et al.</i> (20)	Extension of PK-PD model for artemisinin-based combination therapies (absorption phase of drug concentration-profile included, assessed varying dosing due to age and weight bands and patient adherence)	No estimation of parameters from clinical data Simulated PK and PD curves based on chosen values of PK-PD parameters from other literature. Stochastic simulations ($n=10,000$) performed Simulated PD curves compared with summaries of observed parasitaemia <i>versus</i> time curves from published clinical studies
Stage-specific models		
Gravenor <i>et al.</i> (11)	PD modeling of parasite data from children with cerebral falciparum malaria who received either artemether or quinine	Nonlinear least squares modeling of pooled PD data to determine k_{death1} and k_{death2} (see Fig. 2b), hierarchical nature of the data was ignored. Nonlinear least squares modeling of each individual patient PD profile to determine at the onset of treatment the ratio of the number of parasites aged 1–24 h relative to number aged 25–48 h The PD parameter, PMF, was fixed and thus not estimated from the data The number of parasites in P_1 (parasites aged 1 to 24 h) was fitted to the observed parasitaemia

Svensson <i>et al.</i> (17)	PK-PD modeling of drug concentration and parasite data from adults with uncomplicated falciparum malaria who received the artemisinin combination therapy—artemisinin and mefloquine	Nonlinear mixed-effects modeling of PD data with maximum likelihood estimation (first-order method in NONMEM package) PK data for each individual were simulated from the population PK models derived from nonlinear mixed-effects modeling of the drug concentration data Some of the PD parameters were fixed and thus not estimated from the data Artemisinin assumed to act as an instantaneous mass killing 'pulse' of parasites Between-patient variability was estimated for some of the parameters The total number of parasites in P_{1-2} (parasites aged 1 to 24 h) and the spleen compartment was fitted to the observed parasitaemia
Gravenor <i>et al.</i> (22)	PD modeling of parasite data from children with falciparum malaria who received quinine	Nonlinear least squares modeling of each individual patient PD profile to estimate the parameters (highly unstable-multiple solutions of parameters); followed by nonlinear least squares modeling of pooled PD data to determine stage-specific estimates of k_{death} , hierarchical nature of the data was ignored The PD parameter, PMF, was fixed and thus not estimated from the data The number of parasites in P_{1-24} (parasites aged 1 to 24 h) was fitted to the observed parasitaemia
Gordi <i>et al.</i> (10)	PK-PD modeling of drug concentration and parasite data from adults with uncomplicated falciparum malaria who received artemisinin monotherapy	Nonlinear mixed-effects modeling of PD data with maximum likelihood estimation (first-order method in NONMEM package) PK data for each individual were simulated from the population PK model derived from nonlinear mixed-effects modeling of the drug concentration data Some of the PD parameters were fixed and thus not estimated from the data Between-patient variability was estimated for some of the parameters The total number of parasites in P_1 and P_3 (ring and trophozoite stage of parasite life cycle) and the spleen compartment was fitted to the observed parasitaemia
Hietala <i>et al.</i> (21)	PK-PD modeling of drug concentration and parasite data from children with uncomplicated falciparum malaria who received the artemisinin combination therapy—artemether and lumefantrine	Nonlinear mixed-effects modeling of PD data with maximum likelihood estimation (first-order method in NONMEM package) PK data for each individual were simulated from the population PK models derived from nonlinear mixed-effects modeling of the drug concentration data Some of the PD parameters were fixed and thus not estimated from the data Only between-patient variability was estimated for initial parasitaemia The total number of parasites in P_{1-3} (parasites aged 1 to 15.5 h) and the spleen compartment was fitted to the observed parasitaemia
Discrete-time PK-PD models		
Hoshen <i>et al.</i> (12)	Development of a PK-PD model for artesunate	No estimation of parameters from clinical data Simulated PK and PD curves based on chosen values of PK-PD parameters from other literature. Deterministic simulations performed Simulated PD curves (sum of parasites aged 1 to 24 h) compared with summaries of observed parasitaemia <i>versus</i> time curves from published clinical studies
Smith <i>et al.</i> (16)	PD modeling of parasite data from children with cerebral falciparum malaria who received either artemether or quinine	Nonlinear mixed-effects modeling within a Bayesian framework with informative prior distributions (Metropolis-Hastings algorithm for MCMC in WinBugs package) Simulation study performed to evaluate estimation of the parameter PMF One of the PD parameters was fixed and thus not estimated from the data The total number of parasites in intervals 1 to 6 (parasites aged 1 to 24 h) and 50% of parasites in interval 7 (aged 25 to 28 h) was fitted to the observed parasitaemia
Saralamba <i>et al.</i> (14)	PK-PD modeling of drug concentration and parasite data from adults with uncomplicated falciparum malaria	Monte Carlo simulation of 100,000 sets of the parameters. Pseudo ordinary least squares was used to determine the parameter values that gave the best fit to the data. This

who received artesunate monotherapy
or artesunate plus mefloquine

was done separately for individual observed parasitaemia
versus time curves

The total number of parasites in intervals 1 to 10 (parasites
aged 1 to 10 h) and $2^{\frac{11-a}{3}}$ of parasites in intervals 11 to 48 h
(where a is the age interval of the parasite) was fitted to
the observed parasitaemia

Zaloumis *et al.* (6)

Extension of PK-PD model for
artemisinin-based combination
therapies (absorption phase of drug
concentration-profile included)

No estimation of parameters from clinical data
Simulated PK and PD curves based on chosen values of
PK-PD parameters from other literature and *in vitro* study.
Stochastic simulations ($n=5,000$) performed
Simulated PD curves (sum of parasites aged 1 to 10 h and $2^{\frac{11-a}{3}}$
of parasites aged (a) 11 to 48 h) compared with summaries
of observed parasitaemia *versus* time curves from published
clinical studies

Abbreviations: PK pharmacokinetics, PD pharmacodynamics, PMF parasite multiplication factor

structural and/or deterministic identifiability issues (although a formal analysis was not performed), some model parameters were necessarily held fixed (and specified with reference to the existing literature) in these fitting procedures (Table II).

Smith *et al.* (16) took a Bayesian approach to parameter estimation, using a Markov chain Monte Carlo (MCMC) routine to generate nonparametric posterior distributions (joint distribution of model parameters given the observed data) for all model parameters. The hierarchical nature of the data was taken into account by including all the patient data as part of the same estimation procedure. There are two advantages to this Bayesian approach. First is the use of semi-informative prior distributions (taken from the literature, *e.g.*, modeling of historical syphilis patient data for the distribution of PMF (27,49) and experimental data for killing rate constant of drug (28,44)); which are useful so that we start drawing parameters within the right parameter space (or within a biological plausible range, *e.g.*, for PMF a uniform prior distribution ranging from 1 to 20) and more importantly highlight when we have little data to provide extra information about the parameter (*e.g.*, the resulting posterior distribution is also uniform (1 to 20)). Second, since the method involves taking random draws from the posterior distribution *via* sampling algorithms (*i.e.*, MCMC), the method can be applied to more complex models, helping to alleviate difficulties with model convergence as required for maximum likelihood estimation. Instead, the resulting parameter values from each random draw are assessed visually for convergence and the posterior distribution derived from these random draws (taking the median, 2.5th and 97.5th percentiles) after excluding the initial draws where the parameter value is unstable (*i.e.*, changes greatly between each simulated draw). Whilst powerful, below we discuss both potential difficulties with and solutions to taking this Bayesian MCMC approach to model parameter estimation.

For highly nonlinear parameterised problems which are commonly found in within-host models, current sampling algorithms can be computer intensive and struggle to produce stable posterior distributions of the parameters. The application of parallel tempering (50), in which multiple interacting chains are run, each with a specified “temperature” which compresses the likelihood space and allows the model to more efficiently explore parameter space in search of the global maximum, can provide dramatic improvements in the efficiency of MCMC methods.

DISCUSSION

Within-host anti-malarial PK-PD models provide insight regarding the influence of dosing regimens (amount and frequency of dosing for each anti-malarial) on the parasitological-time profile. These mechanism-based models can be used to simulate hypothetical patient data to inform which treatment strategies should be evaluated in clinical studies. As highlighted in our review, the models have varied structurally, in terms of the incorporation of the life cycle of the falciparum parasite in the red blood cell, the killing action of the drug and the relationship between drug concentration and killing rate constant. Furthermore, validation of the models has differed greatly from the crude approach of simply comparing descriptively the simulated output with published values, to the more robust approach of estimating parameters from clinical data within a Bayesian framework. Mechanistic anti-malarial PK-PD models are a valuable tool in the translational pipeline towards treatment policy, but to date (see section below) their contribution to treatment policy has been under-utilized.

Treatment Policy Contributions of Within-Host Pharmacokinetic-Pharmacodynamic Models

Contribution to treatment policy has primarily relied on findings from population PK studies that have compared the PK profiles of different dosing schemes of an anti-malarial. One of the earliest examples is the change in policy from a single dose of mefloquine (25 mg/kg) to the current recommendation of administering 8.3 mg/kg per day over 3 days with artesunate; evidence for this modification came from population PK modeling of mefloquine drug concentration *versus* time profiles (30). Another example is lumefantrine, where the absorption of the drug was observed to increase if co-administered with fat (51). More recently, population PK modeling has suggested higher doses of oral piperazine (52) and intra-muscular artesunate (53) are required for children; these recommendations are currently being evaluated in clinical studies. Within host PK-PD models have also been used as a decision tool for designing future clinical studies. For example, Saralamba *et al.* simulated PD outcomes for alternative dosing regimens of oral artesunate for treating malaria in areas with emerging artemisinin resistance (14), the proposed dosing regimen (administering every 12 h

Table II. Distribution of Pharmacodynamic Parameters (Parasite Multiplication Factor (PMF) and Killing Rate Constant of Anti-malarial Drug (k_{drug})) Estimated from Clinical Patient Data

	Parasite multiplication factor (PMF) per life cycle (48 h)	Killing rate constant of anti-malarial drug (k_{drug})	
Paper	Population mean (95% reference range) ^a	Population mean (95% reference range) ^a	Further details
Continuous-time PK-PD models			
Gravenor <i>et al.</i> (11)	10 (fixed; NE)	$k_{\text{Artemether}}$: Parasites aged 1–24 h, 3.08 (per 12 h; SE=0.15); Parasites aged 25–48 h, 3.32 (per 12 h; SE=0.37) k_{Quinine} : Parasites aged 1–24 h, 1.98 (per 12 h; SE=0.32); Parasites aged 25–48 h, 3.77 (per 12 h; SE=0.78)	It was assumed $k_{\text{Artemether}}$ and k_{Quinine} did not depend on the drug concentration of artemether and quinine, respectively
Svensson <i>et al.</i> (17)	20 (fixed; NE)	k_{spleen}^b , 0.0913 (per h; 0.0236, 0.353)	$k_{\text{Artemisinin}}$ and $k_{\text{Mefloquine}}$ below represent the combined transfer rate of parasites from each parasite compartment to the spleen compartment— $k_{\text{Artemisinin}}$, 12.1 (per h; NE); $k_{\text{Mefloquine}}$, 0.39 (per nM per h; 0.11, 1.42) It was assumed that $k_{\text{Artemisinin}}$ did not depend on the drug concentration of artemisinin (instead the time above a minimum inhibitory concentration) and $k_{\text{Mefloquine}}$ depended on mefloquine concentration in a linear fashion
Gravenor <i>et al.</i> (22)	12 (fixed; NE)	k_{Quinine} : Parasites aged 36–48 and 1–12 h, 0.035 (per h; SE=0.08); Parasites aged 12–36 h, 0.59 (per h; SE=0.3)	It was assumed k_{Quinine} did not depend on the drug concentration of quinine
Gordi <i>et al.</i> (10)	15 (fixed; NE)	k_{spleen}^b , 0.26 (per h; NE)	$k_{\text{Artemisinin}}$ below represents the combined transfer rate of parasites from each parasite compartment to the spleen compartment— $k_{\text{Artemisinin}}$, 0.45 (per nM per h; NE) It was assumed that $k_{\text{Artemisinin}}$ depended on artemisinin concentration in a linear fashion
Hietala <i>et al.</i> (21)	12 (NE)	k_{spleen}^b , 0.26 (per h; fixed; NE)	$k_{\text{Artemether}}$ below represents the combined transfer rate of parasites from each parasite compartment to the spleen compartment— $k_{\text{Artemether/Dihydroartemisinin}}$, 0.073 (per log _e (nM) ^c per h; NE); $k_{\text{Lumefantrine}}$, NE (NE) It was assumed $k_{\text{Artemether/Dihydroartemisinin}}$ depended on artemether and dihydroartemisinin (both log transformed) in a linear fashion, and that lumefantrine concentrations had no impact on the transfer of parasites to the spleen
Discrete-time PK-PD models			
Smith <i>et al.</i> (16)	Prior distribution—uniform (1,16); posterior distribution, only presented visually. Median between 4 and 8 (derived from Fig. 5 of reference)	$k_{\text{Artemether}}$, 0.38 ^c (per h; NE); k_{Quinine} , 0.36 ^c (per h; NE)	$k_{\text{Artemether}}$ and k_{Quinine} were derived from the maximal proportional reduction in parasites every 4 h time step (defined as M_d , see Eq. 1.6 in reference) ^d ; $M_{\text{Artemether}}$, 0.78 (NE); and M_{Quinine} , 0.76 (NE)
Saralamba <i>et al.</i> (14)	8.8 ^g (4.9–12.7; derived from Table S2 of reference)	$k_{\text{Dihydroartemisinin}}$ —rings, 0.28 ^{f,g} (per h; 0.008, 2.12); trophozoites, 1.51 ^{f,g} (per h; 0.42, 3.01); schizonts, 1.53 ^{f,g} (per h; 0.34, 3.01)	$k_{\text{Dihydroartemisinin}}$ for rings, trophozoites, and schizonts was derived from individual patient estimates of E_{max} (maximum killing effect (% per 48 h), see Table S2 and Eq. S5 of reference) ^d . E_{max} —rings,

84.14^g (% per 48 h; 4.46, 99.99); trophozoites, 99.99^g (% per 48 h; 85.75, 99.99; and schizonts, 99.99^g (% per 48 h; 82.54, 99.99)

Abbreviations: NE not estimated, SE standard error

^a Ninety-five percent of the individual patients have a PMF or k_{drug} within this range

^b A further spleen compartment (which contributes to measured parasitaemia levels) was included in the model as well as the parasite compartments (see Fig. 2c); k_{spleen} relates to the removal of parasites from the system

^c Assumed units since none were given (see Table 3 of reference)

^d Maximum killing effect, concentration *versus* effect relationship assumed to be sigmoidal (see Fig. 1b)

^e Estimated from M_d ($k_{\text{drug}} = \left(\frac{1}{4}\right) \times \log_e \left(\frac{1}{1-M_d}\right)$)

^f Estimated from E_{max} ($k_{\text{Dihydroartemisinin}} = \alpha \times \log_e \left(\frac{100}{100-E_{\text{max}}}\right)$, Eq. S5, Supporting Information of reference)

^g Median (2.5th–97.5th percentiles)

instead of daily) was evaluated in a clinical trial in South East Asia (54).

Extending the Pharmacokinetic-Pharmacodynamic Models

Our review of the literature highlights two areas where existing PK-PD models do not accurately reflect our evolving understanding of the host-pathogen-drug system. Firstly, with the exception of Austin *et al.* (9) and in the supplementary information of Winter and Hastings (5), models of the parasite population dynamics fail to adequately account for the parasite-growth limiting effects of the host's natural defense mechanisms, mediated through the innate and adaptive immune responses. Reduced host resource availability (*i.e.*, depleted erythrocyte levels), whilst capable of reducing parasite growth, is unlikely to contribute as strongly, if at all, as immune responses. Khoury *et al.* observed from modeling of data from mice infected with *Plasmodium berghei* that the slowing down of parasite growth was not due to the host running out of red cells for the parasite to invade (55). Instead, most of the published models assume continual exponential growth of the parasite load in the absence of anti-malarial drugs. Many have argued that this assumption is justified because the aim of the PK-PD model is to derive anti-malarial dosing schemes that are effective for all patient populations, especially non- and partially immune young children who account for 58% of deaths due to malaria (in 2010) (56). However, when fitting such models to data from treated patients with follow-up longer than 1–2 weeks, estimates of drug effect would be expected to be systematically overestimated as drug killing *in the model* must overcome continued natural exponential growth, while in reality, other host-mediated responses would be naturally limiting parasite load. Winter and Hastings (5) simulated longitudinal parasite-count data with long term follow-up to derive whether a patient from a hypothetical clinical trial was cured or failed treatment. The PK-PD model was expanded to include an empirical measure of immunity that was dependent on the entomological inoculation rate (EIR). Hypothetical patients assigned to be residing in an area with a high EIR were associated with a reduced odds of failure, illustrating that modeled immunity influences the calculated likelihood of failure, which is the common clinical outcome measure used for patients with uncomplicated malaria. As for other host-pathogen systems (*e.g.*, influenza (45)) the challenge remains to construct models of increased biological fidelity which nonetheless are structurally identifiable and for which model parameters may be robustly and precisely estimated.

Secondly, there is emerging evidence (28,57) that the killing action of the current front-line anti-malarials, the artemisinin derivatives, are not accurately described by a classic PK-PD relationship (see Fig. 1a–c). Under the standard PK-PD paradigm, the parasite killing rate at time t is a function of the drug concentration at time t . However, the data of Klonis *et al.* (28) provide compelling evidence, albeit in the *in vitro* setting, that the killing rate of parasites at a given time depends upon not only the current drug concentration, but the drug exposure history of the parasites under study. Patel *et al.* also showed, in mice inoculated with *P. berghei* malaria, that the parasites need to be exposed to dihydroartemisinin for at least 5 h before killing occurs (57). Some of the present authors, in contributing to the findings in Klonis *et al.*, have developed a semi-mechanistic model to explain the *in vitro* observations, but it remains an important and open question as to how to appropriately incorporate these drug accumulation effects into the within-host PK-PD models. Of note, Smith *et al.* (16) allowed for a delay in the drug effect of artemisinin and quinine of 3 to 4 h based on findings from *in vitro* data (58).

Balancing Model Complexity With Statistical Rigor

As in all mechanistically motivated modeling studies (of a biological nature or otherwise) models should be developed with a particular purpose and scientific question in mind (46). Reflecting this, there is significant variation in the level of biological fidelity incorporated in different within-host malaria models. Importantly, it should not be assumed that with the passage of time, increasing model complexity is either desired or inevitable. Models must be *fit for purpose*. Models must be *falsifiable*. Prior to the fitting of models to clinical data, models should be evaluated to determine how sensitive model outputs (*e.g.*, parasite clearance time and cure) are to model inputs (*e.g.*, PMF, k_{drug}). This can be done through simulation studies, ideally in a full multivariate framework (*e.g.*, Latin Hypercube Sampling (6)), whereby all input parameters are (co-)varied and associations between outputs and inputs assessed both visually and using statistical measures such as correlation and regression. Following this evaluation of the structural identifiability of the PK-PD model, it is important next to determine if the parameters can be estimated from the available clinical data (*i.e.*, the deterministic identifiability of the model). This can be explored by performing a simulation-estimation exercise, where parameters are estimated from simulated data, starting with rich parasitaemia *versus* time sampling designs followed by sampling designs (*e.g.*, six hourly or daily

measurements of parasite count) that correspond to the clinical data available.

Future population PK-PD studies should be designed to ensure that there is optimal sampling in terms of the number and timing of blood samples collected (for measurement of blood drug concentrations and parasite burden) to provide sufficient information for precise estimation of the PK and PD profiles. We have recently used optimal design theory (a model based data-independent approach) to provide optimal sampling windows for population PK studies of ACTs (33,34) and for an empirical measure of parasite clearance (59). To date, optimal sampling designs have not been presented in the literature for PK-PD studies where one of the aims of the study is to evaluate complex mechanistic anti-malarial PK-PD models. Our above suggestion of a model-based data-dependent approach (*i.e.*, simulation-estimation exercise) would provide insight into sampling designs of future PK-PD studies of both current and new anti-malarial therapies.

CONCLUSIONS

In summary, anti-malarial PK-PD models are powerful tools to investigate the impact of different anti-malarial dosing regimens and combination therapies on parasite dynamics. Clinical trials are expensive compared with simulations derived from mechanism-based models. We recommend using modeling to inform which anti-malarial treatments can then be evaluated prospectively in rigorously designed clinical evaluations. As highlighted in our review, the utility of these models depends on how well the predicted parasite *versus* time profiles match the patient data. We recommend a thorough evaluation of the structural and deterministic identifiability of the PK-PD models prior to fitting the model to clinical data. Following this, we propose fitting the models to clinical data within a Bayesian framework to improve parameter estimation.

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