Predicting the parasite killing effect of artemisinin combination therapy in a murine malaria model

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Objectives: To develop a mechanism-based model that describes the time course of the malaria parasite in infected mice receiving a combination therapy regimen of dihydroartemisinin and piperaquine.

Methods: Total parasite density–time data from Swiss mice inoculated with Plasmodium berghei were used for the development of population models in S-ADAPT. The mice were administered a single intraperitoneal dose of 30 mg/kg dihydroartemisinin, 10 mg/kg piperaquine phosphate or a combination of both antimalarials at 64 h post-inoculation. In a separate study, mice received multiple dihydroartemisinin doses (5 × 10 mg/kg or 30 mg/kg dihydroartemisinin followed by two 10 mg/kg doses). Parasite recrudescence after treatment was defined using a model that incorporated each erythrocytic stage of the P. berghei life cycle.

Results: The disposition of dihydroartemisinin and piperaquine was described by a one-compartment and two-compartment model, respectively. The estimated clearance was 1.95 L/h for dihydroartemisinin and 0.109 L/h for piperaquine. A turnover model described the parasite killing curve after single-agent dosing, with an estimated mean IC50 of 0.747 μg/L for dihydroartemisinin and 16.8 μg/L for piperaquine. In addition, the rate of parasite killing by dihydroartemisinin was almost 50-fold faster than for piperaquine. Parameters from the monotherapy models adequately described the parasite density–time curve following dihydroartemisinin/piperaquine combination therapy or multiple-dose regimens of dihydroartemisinin.

Conclusions: This study has developed mechanistic models that describe the parasite–time curve after single, multiple or combination dosing of antimalarials to mice. These structural models have potential application for pre-clinical investigations to design and refine artemisinin-based combination therapy dosage regimens.

Keywords: dihydroartemisinin, mechanism-based modelling, piperaquine, Plasmodium berghei, population pharmacodynamics

Introduction

Artemisinin-based combination therapy (ACT) is currently recommended as first-line treatment of falciparum malaria in all patient populations. This regimen combines artemisinin derivatives (artesunate, artemether or dihydroartemisinin) with another antimalarial drug such as piperaquine, mefloquine, amodiaquine or lumefantrine. The artemisinins significantly reduce the parasite bio-burden in the first 24–48 h following treatment and are more potent than other available antimalarial agents. However, the current generation of artemisinin derivatives in clinical use are rapidly eliminated (t1/2 ~1 h) and are therefore unable to eradicate any residual parasites after the typical 3 day treatment regimen. Co-administration with partner drugs that have a longer duration of action is therefore suggested to offset parasite recrudescence after artemisinin dosing. Thus, ACT is potentially curative because malaria parasites are effectively eliminated in the infected patient. Despite the promise of ACT, optimum dosing schedules are difficult to establish and currently poorly defined. This is not unexpected, given the ethical and logistical constraints associated with the clinical determination of antimalarial plasma or blood concentrations and parasite burden. A further drawback is that the total parasite burden is not measurable in humans with falciparum malaria, since only parasites circulating in the blood are detected by microscopy. These issues may limit the design of effective ACT regimes, which must incorporate the rates of parasite killing by individual antimalarials and the overall effect of different drug combinations (i.e. additive or synergistic effects).
One possible solution is the development of mechanistic mathematical models to facilitate better ACT dosing guidelines. These models describe the time course of parasite growth, antimalarial drug concentration (pharmacokinetics; PK) and their corresponding rates of parasite killing (pharmacodynamics; PD). The resulting parameters of interest can then be used to simulate the expected parasite kill curve following various schedules and doses of monotherapy or combination therapy regimens. This approach is efficient, cost-effective and can support the design of ACT recommendations that are likely to remove the majority of malaria parasites from the infected host. 

Recently, we have reported a mechanistic model that describes the growth dynamics of Plasmodium berghei and the effect of dihydroartemisinin in murine malaria. In this study, we adapt the model to estimate total parasite killing after single- or multiple-dose dihydroartemisinin and single-dose piperaquine. Ultimately, we show that understanding of the PK and PD of individual antimalarials can successfully predict parasitcidal activity following multiple dosing regimens or combination therapy.

### Methods

#### Source of antimalarial data

Parasite density-versus-time data were obtained from previous studies in male Swiss mice (aged 5–6 weeks; average weight 29.5±3.3 g) infected with 10^7 P. berghei parasitized erythrocytes. Groups of mice received a single intraperitoneal (ip) dose of 30 mg/kg dihydroartemisinin (n = 15), 10 mg/kg piperaquine phosphate (n = 13) or a combination of both antimalarials (n = 16) at 64 h after inoculation (parasitaemia, 3%–5%). Tail vein bleed were performed at 64, 68, 72, 76, 88, 92, 96, 100, 112, 116, 120, 136, 160, 164, 190, 232, 253 and 280 h post-inoculation. The parasitaemia was determined using light microscopy of peripheral blood smears, and a linear relationship was used to convert the total percentage of infected erythrocytes into a parasite density (detection limit 1000 parasites/μL). In a separate and unreported pilot study using established methods, two cohorts of infected mice were used to investigate parasite killing after multiple doses of dihydroartemisinin. The first cohort (n = 8) received five doses of 10 mg/kg dihydroartemisinin every 12 h and the second group (n = 8) were given a single dose of 30 mg/kg dihydroartemisinin followed by two 10 mg/kg doses every 12 h. Animals were treated by ip injection at 72 h after inoculation of P. berghei erythrocytes to non-infected mice. Blood was harvested at 72, 74, 76, 78, 82, 84, 96, 98, 100, 102, 104, 108, 120, 124, 126, 129, 135, 147, 151, 173, 245, 269 and 293 h for the determination of parasitaemia.

Plasma concentration–time data for dihydroartemisinin or piperaquine were obtained from earlier reports. In these studies, infected mice received a single ip dose of 100 mg/kg dihydroartemisinin (n = 32) or 90 mg/kg piperaquine (n = 91). Blood was collected by cardiac puncture at 2, 5, 10, 15, 30, 45, 60, 90 and 120 min (dihydroartemisinin dosing), and 2, 4, 6, 12, 18, 24, 30, 48, 56, 72, 96, 120, 168, 216, 360 and 528 h (piperaquine dosing). Plasma samples were analysed using HPLC. The modified limit of quantification (LOQ) in mouse plasma was 90 μg/L for dihydroartemisinin and 1.5 μg/L for piperaquine, with a precision of 9% for both assays. All animal studies were approved by the Curtin University of Technology Animal Ethics Committee and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes.

#### Mathematical modelling

Logarithmically transformed parasite density–time profiles were combined with PK data and simultaneously analysed by non-linear mixed effects modelling using S-ADAPT (version 1.57) and the SADAPT-TRAN facilitator tool. Model selection was based on the visual inspection of diagnostic scatter plots, the biological plausibility of parameter estimates and the objective function value (OBJ; reported as −1×log likelihood in S-ADAPT). A decrease in the OBJ of 1.92 U (α = 0.05) for nested models was considered statistically significant. A well-established likelihood-based (M3) method was applied to data below the LOQ. The between-subject variability (BSV) was assumed to follow a lognormal distribution, with the magnitude reported as an apparent coefficient of variation (CV). Residual unexplained variability (RUV) in the PK data was defined using a combination of additive and exponential random error. The RUV for logarithmically transformed parasite density profiles was calculated using an exponential error model.

### Defining the parasite life cycle

Previously, we developed a mechanism-based model that explicitly described the life cycle for each of the erythrocytic stages (ring, early trophozoite, late trophozoite and schizont) of P. berghei. To retain the structure of our mechanism-based growth model (Figure 1), stage-specific data were additionally incorporated into the present analysis. Changes in the numbers of parasitized erythrocytes after ip inoculation (dose = 10^7 parasites) were calculated using the first-order rate constant for the absorption of parasitized erythrocytes (kabs) and the bioavailability for rings (freg), early trophozoites (fetrop) and late trophozoites (fetrop). The number of schizonts in the initial inoculum was assumed to be negligible. The model also allowed for the simultaneous estimation of P. berghei recrudescence, immune elimination and sequestration. However, as with most studies of antimalarial efficacy, the data used in the current study represented total parasite density and did not classify each of the erythrocytic stages of the parasite life cycle. Total parasite density (Cpar;tot) was calculated by summing the predicted amounts for each individual erythrocytic stage and dividing by the estimated volume of parasite distribution (Equation 1).

\[ C_{\text{par;tot}} = \frac{(A_{\text{ring}} + A_{\text{etrop}} + A_{\text{ltrop}} + A_{\text{schiz}})}{V_{\text{pc}}}, \]  

where \( A_{\text{ring}}, A_{\text{etrop}}, A_{\text{ltrop}} \) and \( A_{\text{schiz}} \) are the amounts of rings, early trophozoites, late trophozoites and schizonts, respectively, and \( V_{\text{pc}} \) is the estimated volume of parasite distribution (see Table 2).

The estimation of total parasite density by this method gave results that were comparable to observed data, thereby validating the above approach. A further advantage of using the above structural model is that it provides the ability for exploring the stage specificity of antimalarial drugs.

### PK and PD modelling

One-, two- and three-compartment models were evaluated to describe the disposition of dihydroartemisinin or piperaquine in plasma. Drug input from the ip dosing depot into the central compartment was tested using zero- or first-order absorption kinetics. For the estimation of residual error, the exponential and additive components were fixed to assay precision and LOQ, respectively, since only a single plasma observation was obtained from each mouse. The resulting PK models were then used to simultaneously define the relationship between drug concentration and parasite killing.

The effect of antimalarial-induced parasite elimination was characterized by a turnover model, in which drug inhibited the production of hypothetical physiological processes. For this, a turnover compartment was used to describe changes in the amount of physiological intermediate (Aphys). Characteristically, this allows for the development of a biologically plausible model that describes the delay between the maximum drug concentration and the maximal response (parasite nadir in the current study).
The PD effect was then modelled as shown in Equation 2:

$$\frac{dA_{phys}}{dt} = k_\text{in} \cdot \left(1 - \frac{C_{drug}}{IC_{50} + C_{drug}}\right) - k_{out} \cdot A_{phys}$$

(2)

where $k_{in}$ is the zero-order rate of $A_{phys}$ production, $C_{drug}$ is the drug concentration in plasma, $IC_{50}$ is the drug concentration at half-maximal $A_{phys}$ inhibition and $k_{out}$ is the first-order rate constant for $A_{phys}$ loss. At initial steady-state conditions ($IC$), the rate of $A_{phys}$ production is equal to that of its degradation ($k_{in}/k_{out} = 1$). The apparent lack in $A_{phys}$ is:

$$lack_{phys} = 1 - A_{phys}$$

(3)

For each intra-erythrocytic stage ($A_{par\_stage}$), the rate of parasite killing was generically described as:

$$\frac{dA_{par\_stage}}{dt} = -k_{drug} \cdot \left(\frac{lack_{phys}}{kill_{50} + lack_{phys}}\right) \cdot A_{par\_stage}$$

(4)

where $k_{drug}$ is the first-order rate constant for parasite elimination by drug and $kill_{50}$ is the amount of physiological intermediates at half-maximal killing. Equation 4 ensures zero killing of parasite in the absence of drug, where $A_{phys}$ is arbitrarily defined at a steady-state of 1 (i.e. $lack_{phys} = 0$). A schematic of the parasite killing (turnover) model is illustrated in Figure 2. For each antimalarial drug, a unique set of PD parameters was
estimated after single-agent dosing. In the combined therapy model, the rate of stage-specific parasite killing was defined by summing the individual rates obtained after monotherapy of dihydroartemisinin or piperaquine (Equation 5).

\[
\frac{dA_{\text{par}_{\text{stage}}}}{dt} = -\left[ k_{\text{DHA}} \cdot \left( \frac{\text{lack}_{\text{phys}}}{\text{kill}_{50_{\text{DHA}}} + \text{lack}_{\text{phys}}} \right) + k_{\text{PQ}} \cdot \left( \frac{\text{lack}_{\text{phys}}}{\text{kill}_{50_{\text{PQ}}} + \text{lack}_{\text{phys}}} \right) \right] \cdot A_{\text{par}_{\text{stage}}}
\]

(5)

All models were evaluated by a visual predictive check, for which 1000 datasets were simulated from the final parameter estimates using the original data as a template. The median, 25th and 75th percentiles of simulated predictions were then calculated and plotted against observed values.

Table 1. Population PK parameter estimates for dihydroartemisinin and piperaquine in malaria-infected mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Population estimate (BSV, CV%) (^\text{a})</th>
<th>dihydroartemisinin</th>
<th>piperaquine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D1) (h)</td>
<td>zero-order absorption duration</td>
<td>0.212 (47.8)</td>
<td>0.654 (61.3)</td>
<td></td>
</tr>
<tr>
<td>(CL/F) (L/h)</td>
<td>central compartment clearance</td>
<td>1.95 (8.20)</td>
<td>0.109 (54.6)</td>
<td></td>
</tr>
<tr>
<td>(Vc/F) (L)</td>
<td>central volume of distribution</td>
<td>0.851 (26.6)</td>
<td>21.8 (71.4)</td>
<td></td>
</tr>
<tr>
<td>(CLD/F) (L/h) (^\text{b})</td>
<td>inter-compartmental clearance</td>
<td>—</td>
<td>0.729 (64.2)</td>
<td></td>
</tr>
<tr>
<td>(Vp/F) (L) (^\text{b})</td>
<td>peripheral volume of distribution</td>
<td>—</td>
<td>33.5 (34.5)</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Between-subject variability of magnitude % coefficient of variation.

\(^{b}\)Not required for dihydroartemisinin because the disposition was adequately described using a one-compartment model.

Results

PK models for dihydroartemisinin and piperaquine

The plasma disposition of dihydroartemisinin and piperaquine in malaria-infected mice was described by one- and two-compartment models, respectively. For dihydroartemisinin, the estimated mean clearance from the central compartment was 1.95 L/h (BSV 8.2%), while that for piperaquine was 0.109 L/h (BSV 54.6%). Steady-state volumes of distribution were 0.85 L for dihydroartemisinin and 55.3 L for piperaquine. The ip absorption of both compounds was best described by zero-order absorption kinetics. A summary of population PK parameter estimates is provided in Table 1, and Figure 3 shows the visual predictive check for both antimalarial agents.

Parasite kill model after monotherapy

A turnover model described the parasite kill curve after single-agent dihydroartemisinin or piperaquine dosing. In these turnover models, both antimalarials inhibited the production of hypothetical physiological intermediates. The estimated \(IC_{50}\) was 0.747 mg/L (BSV 40.5%) for dihydroartemisinin and 16.8 mg/L (BSV 5.10%) for piperaquine. In addition, the rate of drug-induced parasite killing (described by Equation 4) was almost 50-fold slower for piperaquine (at the same \(lack_{\text{phys}}\) for both drugs). Furthermore, the onset of parasite elimination by piperaquine was observed and predicted to occur \(\approx 12\) h after drug dosing (Figure 4, right panel between 64 h and 76 h post-inoculation). By contrast, the decline in total parasite density was apparent between 64 h and 66 h following dihydroartemisinin treatment (Figure 4, left panel). Figure 4 also demonstrates that parasite recrudescence (after 90 h for dihydroartemisinin and 120 h for piperaquine) was suitably described by our previous \(P.\) berghei growth model.\(^\text{19}\)

Antimalarial stage specificity

During model development, the antimalarial effect was sequentially explored for each of the individual erythrocytic stages (ring, late trophozoite and schizont) of \(P.\) berghei development.
A key finding was that the parasite kill curve after dihydroartemisinin dosing was appropriately described only when the turnover model was simultaneously applied to all erythrocytic stages. This result is illustrated by simulation (Figure 5), whereby the parasite nadir occurred later than observed data when the drug effect was included only on rings or trophozoites or schizonts. A correction of this rightward shift occurred when the dihydroartemisinin effect was incorporated in all stages of the \textit{P. berghei} life cycle. For piperazine, adequate prediction of the parasite time course only required application of the PD (turnover) effect on trophozoites and schizonts (data not shown).

The PD parameter estimates for dihydroartemisinin monotherapy were subsequently used to describe \textit{P. berghei} elimination after multiple dosing [10 mg/kg every 12 h (five doses) or 30 mg/kg dihydroartemisinin followed by two 10 mg/kg doses every 12 h]. The same approach was applied to the prediction of the parasite density–time course after single-dose combination therapy (30 mg/kg dihydroartemisinin and 10 mg/kg piperazine phosphate). For each of the above models, the population mean values were fixed to those reported in Table 2, with BSV estimated on these parameters. Good predictive performance of the model was demonstrated after multiple dosing of dihydroartemisinin (Figure 6, left panel) or the single-dose combination of dihydroartemisinin plus piperazine (Figure 6, right panel). The BSV estimates for all PD model parameters ranged from 3.1% to 25.1%, with a 16.8% exponential error. Model predictions for the combination of dihydroartemisinin/piperazine demonstrated an initial rapid rate of parasite kill (Figure 6, right panel), which was primarily attributed to dihydroartemisinin, and a nadir that was comparable following dihydroartemisinin monotherapy (Figure 4, left panel). As expected for ACT, a slower rate of recrudescence was predicted relative to dihydroartemisinin alone (due to the synergistic effect of piperazine) and this finding was consistent with the original study. Simulated profiles showed that the lower dose and longer \(\frac{t_1}{t_2}\) of piperazine allows for the clearance of residual parasites during recrudescence (data not shown), which is the overall aim of ACT. However, the absence of a biphasic distribution in the parasite–time profile during ACT is likely to occur because of the higher IC\(50\) and slower rate of piperazine killing relative to that for dihydroartemisinin.

**Prediction of multiple dosing and combination therapy**

The PD parameter estimates for dihydroartemisinin monotherapy were subsequently used to describe \textit{P. berghei} elimination after multiple dosing [10 mg/kg every 12 h (five doses) or 30 mg/kg dihydroartemisinin followed by two 10 mg/kg doses every 12 h]. The same approach was applied to the prediction of the parasite density–time course after single-dose combination therapy (30 mg/kg dihydroartemisinin and 10 mg/kg piperazine phosphate). For each of the above models, the population mean values were fixed to those reported in Table 2, with BSV estimated on these parameters. Good predictive performance of the model was demonstrated after multiple dosing of dihydroartemisinin (Figure 6, left panel) or the single-dose combination of dihydroartemisinin plus piperazine (Figure 6, right panel). The BSV estimates for all PD model parameters ranged from 3.1% to 25.1%, with a 16.8% exponential error. Model predictions for the combination of dihydroartemisinin/piperazine demonstrated an initial rapid rate of parasite kill (Figure 6, right panel), which was primarily attributed to dihydroartemisinin, and a nadir that was comparable following dihydroartemisinin monotherapy (Figure 4, left panel). As expected for ACT, a slower rate of recrudescence was predicted relative to dihydroartemisinin alone (due to the synergistic effect of piperazine) and this finding was consistent with the original study. Simulated profiles showed that the lower dose and longer \(\frac{t_1}{t_2}\) of piperazine allows for the clearance of residual parasites during recrudescence (data not shown), which is the overall aim of ACT. However, the absence of a biphasic distribution in the parasite–time profile during ACT is likely to occur because of the higher IC\(50\) and slower rate of piperazine killing relative to that for dihydroartemisinin.

**Discussion**

This study demonstrated a unique series of models that describe the time course of antimalarial concentration, parasite elimination after treatment and subsequent recrudescence in mice infected with \textit{P. berghei}. The models were developed using the structure of our recently reported mechanism-based murine model, which quantitatively details the \textit{P. berghei} life cycle for each erythrocytic stage, immune elimination and dihydroartemisinin-induced parasite killing. Here, we further adapted this model to the estimation of total parasite density data after dihydroartemisinin or piperazine monotherapy. The results were then used to predict parasite
killing and recrudescence following dihydroartemisinin multiple dosing, or a single-dose dihydroartemisinin/piperaquine combination that was designed to be sub-therapeutic (not curative) in order to provide rich data for PK–PD modelling. The present study supports the use of mechanistic mathematical modelling to assist with the rational development of ACT dosing guidelines, as recommended by the WHO.8

Population PK analysis of dihydroartemisinin and piperaquine in plasma were characterized by one- and two-compartment models, respectively (Table 1). The disposition of both antimalarial drugs was comparable to previous reports in mice, 20,22,29 with elimination $t_1/2$ values for dihydroartemisinin and piperaquine of $\sim 0.3$ h and 350 h respectively.

The present analysis describes a delay between the maximal drug concentration and the parasite nadir, which is a novel advance in models for antimalarial activity.16,30 This turnover model inhibited the production of hypothetical physiological intermediates, and includes all of the likely reported mechanisms of action for dihydroartemisinin activity.31–33 The estimated $IC_{50}$ values (0.75 mg/L for dihydroartemisinin and 16.8 mg/L for piperaquine; Table 2) are consistent with data from in vitro experiments.34–38 Furthermore, the overall rate of parasite killing by dihydroartemisinin was faster than for piperaquine, as has been described previously.4,39 The $kill_{50}$ parameter simply provides a saturable rate of drug-induced parasite elimination that statistically improved the model fit. It is pharmacologically less relevant than the $IC_{50}$, which is a measure of drug potency. A further benefit of the mechanism-based model is the ability to dissect the stage specificity of antimalarial effects. In the present study, an adequate fit of the parasite kill curve after dihydroartemisinin

### Table 2. Population parameter estimates for the mechanism-based model describing the life cycle of *P. berghei* and the parasite killing effect of dihydroartemisinin (DHA) and piperaquine (PQ)

| Kinetic process | Parameter | Description | Unit | Estimate (%RSE)$^b$ | BSV (CV%)$^c$
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasite absorption$^a$</td>
<td>$T_{absop}$</td>
<td>mean time for parasite absorption</td>
<td>h</td>
<td>0.0522 (35.7)</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td>$F_{ring}$</td>
<td>bioavailability of rings</td>
<td>—</td>
<td>0.568 (64.6)</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>$F_{trp}$</td>
<td>bioavailability of early trophozoites</td>
<td>—</td>
<td>0.101 (28.4)</td>
<td>27.4</td>
</tr>
<tr>
<td></td>
<td>$V_{bc}$</td>
<td>volume of parasite distribution</td>
<td>$\mu$L</td>
<td>0.0289 (7.12)</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>$F_{trp}$</td>
<td>bioavailability of late trophozoites</td>
<td>—</td>
<td>1.180 (4.54)</td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>$MF$</td>
<td>number of merozoites per schizont</td>
<td>—</td>
<td>2.20 (1.46)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$T_{inf}$</td>
<td>mean time for infection of erythrocytes</td>
<td>h</td>
<td>0.0446 (17.2)</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>$A_{M50}$</td>
<td>log$_{10}$ of merozoites at half-maximal infection decline</td>
<td>—</td>
<td>5.86 (1.46)</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>$inhill$</td>
<td>Hill coefficient for decline in infection</td>
<td>—</td>
<td>0.766 (1.92)</td>
<td>—</td>
</tr>
<tr>
<td>$P. berghei$ life cycle$^a$</td>
<td>$T_{ring}$</td>
<td>mean time for rings transfer</td>
<td>h</td>
<td>1.10 (5.49)</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>$T_{trp}$</td>
<td>mean time for early trophozoite transfer</td>
<td>h</td>
<td>3.31 (4.41)</td>
<td>16.3</td>
</tr>
<tr>
<td></td>
<td>$T_{sch}$</td>
<td>mean time for schizont transfer</td>
<td>h</td>
<td>16.6 (2.86)</td>
<td>5.54</td>
</tr>
<tr>
<td></td>
<td>$MF$</td>
<td>mean time for elimination of merozoites per schizont</td>
<td>—</td>
<td>3.60 (2.88)</td>
<td>9.18</td>
</tr>
<tr>
<td>Merozoite infection$^a$</td>
<td>$A_{M50}$</td>
<td>log$_{10}$ of merozoites at half-maximal infection</td>
<td>—</td>
<td>5.86 (1.46)</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>$inhill$</td>
<td>Hill coefficient for decline in infection</td>
<td>—</td>
<td>0.766 (1.92)</td>
<td>—</td>
</tr>
<tr>
<td>Parasite elimination$^a$</td>
<td>$T_{xs}$</td>
<td>mean time for late trophozoite elimination</td>
<td>h</td>
<td>2.390 (31.9)</td>
<td>43.6</td>
</tr>
<tr>
<td></td>
<td>$T_{x}$</td>
<td>mean time for elimination of schizonts</td>
<td>h</td>
<td>115 (37.2)</td>
<td>25.2</td>
</tr>
<tr>
<td></td>
<td>$T_{s}$</td>
<td>mean time for elimination of merozoites</td>
<td>h</td>
<td>5.99 (12.0)</td>
<td>15.1</td>
</tr>
<tr>
<td>Schizont efflux$^a$</td>
<td>$V_{sch}$</td>
<td>log$_{10}$ of maximal schizonts efflux per hour</td>
<td>—</td>
<td>7.31 (0.132)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$A_{x50}$</td>
<td>log$_{10}$ of schizonts at half-maximal efflux</td>
<td>—</td>
<td>6.95 (0.321)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>$MT_{trans}$</td>
<td>mean transit time of schizonts</td>
<td>h</td>
<td>7.49 (11.3)</td>
<td>39.8</td>
</tr>
<tr>
<td>DHA PD$^a$</td>
<td>$IC_{50}$</td>
<td>DHA concentration at 50% killing</td>
<td>ng/mL</td>
<td>0.747 (35.8)</td>
<td>40.5</td>
</tr>
<tr>
<td></td>
<td>$K_{II}$</td>
<td>lack of physiological intermediate at 50% kill by DHA</td>
<td>—</td>
<td>0.0578 (44.3)</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>$T_{DHA}$</td>
<td>mean time for parasite elimination by DHA</td>
<td>h</td>
<td>5.43 (17.5)</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>$T_{eff}$</td>
<td>half-time for elimination of DHA turnover model</td>
<td>h</td>
<td>2.89 (12.1)</td>
<td>6.9</td>
</tr>
<tr>
<td>PQ PD$^a$</td>
<td>$IC_{50}$</td>
<td>PQ concentration at 50% killing</td>
<td>ng/mL</td>
<td>16.8 (36.7)</td>
<td>5.10</td>
</tr>
<tr>
<td></td>
<td>$K_{II}$</td>
<td>lack of physiological intermediate at 50% kill by PQ</td>
<td>—</td>
<td>1.36 (24.1)</td>
<td>3.90</td>
</tr>
<tr>
<td></td>
<td>$T_{PQ}$</td>
<td>mean time for parasite elimination by PQ</td>
<td>h</td>
<td>1.05 (18.3)</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>$T_{eff}$</td>
<td>half-time for elimination of PQ turnover model</td>
<td>h</td>
<td>8.40 (22.5)</td>
<td>7.30</td>
</tr>
<tr>
<td>Residual error</td>
<td>$SD_{exp,DHA}$</td>
<td>exponential error for DHA model</td>
<td>CV%</td>
<td>—</td>
<td>16.9</td>
</tr>
<tr>
<td></td>
<td>$SD_{exp,PQ}$</td>
<td>exponential error for PQ model</td>
<td>CV%</td>
<td>—</td>
<td>24.9</td>
</tr>
</tbody>
</table>

$^a$Defined using data and model for individual erythrocytic stages.19

$^b$%RSE, % relative standard error.

$^c$CV%, % coefficient of variation for between-animal variability.

$^d$All first-order rate constants ($k$) were parameterized and estimated as mean times ($1/k$) to facilitate the interpretation of parameter estimates.

$^e$Described using total parasite density data from current study.
dosing was only provided when the PD model was simultaneously applied to all intra-erythrocytic stages of the *P. berghei* life cycle. By contrast, the elimination of parasite by piperaquine required application of the PD effect to trophozoites and schizonts only. These findings are also consistent with established principles of antimalarial efficacy, whereby artemisinin drugs have broad stage specificity, but quinolines such as piperaquine are most active against mid–late intra-erythrocytic stages of the parasite. 39–43

A limitation in the PK–PD model was the inability to accurately estimate the ‘true’ IC50 of drug. This was due to the high potency of the drugs, whereby the ‘sub-therapeutic’ doses used in the experiments actually produced plasma concentrations that were well above the estimated IC50. For dihydroartemisinin, *in vivo* estimates of IC50 did not change significantly when sequentially testing (Figure 5) the drug–killing model on each of the erythrocytic stages of *P. berghei* (IC50 range: 1.46–1.73 μg/L). In order to properly identify the IC50, further experimentation at lower doses would be useful. This is a key study design recommendation when testing the differential susceptibility of erythrocytic stages to antimalarial drugs.

Based on results from the individual drugs, modelling parasite elimination after the dihydroartemisinin/piperaquine combination adequately described the central tendency and variability in the data (Figure 6). Thus, we have demonstrated that a detailed understanding of the PK–PD for individual antimalarial agents can be used to predict the effect of multiple or combination treatment regimens, which for dihydroartemisinin/piperaquine reflects the clinical efficacy reported in clinical studies. 44,45 Hence, a simulation platform could be used to develop or refine dosing schedules and drug combinations for clinical trials. Animal models of malaria infection are particularly useful in this regard, because intensive sub-therapeutic and curative PK–PD data are accessible, and measurement can be classified into individual stages of the parasite life cycle. 46–48 While researchers must be cognizant of differences between mice and humans, these are primarily associated with disease pathophysiology and there are potential benefits for using murine models of infection. 49,50 To our knowledge, sophisticated translation of drug PK–PD from pre-clinical species to humans is limited in malaria research. For the parasite–time profile, the main consideration is the duration of asexual schizogony, which takes 18–24 h for rodent *P. berghei* and ~48 h for human *Plasmodium falciparum*. 48,50 Further work in this area is therefore required to establish whether the PK–PD from pre-clinical models can be appropriately translated into human prediction. This advance may be particularly useful because of the known limitations in the collection of clinical blood samples for the determination of antimalarial concentrations and parasite burden, especially in children. 10–12

In conclusion, we have developed mechanistic models that describe the parasite–time curve after single, multiple or combination dosing of antimalarials in malaria-infected mice. This modelling also predicts total parasite density and recrudescence using established parameters for the *P. berghei* life cycle. These structural models can be applied to the pre-clinical development of novel antimalarial agents or other emerging artemisinin analogues. Ultimately, we propose that this model can assist with the efficient clinical development of ACT dosing recommendations.

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**Transparency declarations**

None to declare.

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