Incorporating Stage Specific Drug Action into Pharmacological Modelling of Antimalarial Drug Treatment

Eva Maria Hodel, Katherine Kay, Ian M Hastings

Liverpool School of Tropical Medicine, Liverpool, United Kingdom

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Abstract

Pharmacological modelling of anti-parasitic treatment based on a drug's pharmacokinetic and pharmacodynamic properties plays an increasingly important role in identifying optimal drug dosing regimens and predicting their potential impact in control and elimination programmes. Conventional modelling of treatment relies on methods that do not distinguish between parasites being in different developmental stages. This is problematic for malaria parasites as their sensitivity to drugs varies substantially during their 48-hour developmental cycle. We investigated four drug types (short/long half-lives with/without stage specific killing) to quantify the accuracy of the standard methodology. The treatment dynamics of three drug types were well characterised with standard modelling. The exception were short half-life drugs with stage specific killing (i.e. artemisinins) because, depending on time of treatment, parasites might be in highly drug-sensitive stages or in much less sensitive stages. We describe how to bring such drugs into pharmacological modelling by including additional variation into the drugs maximal killing rate. Finally, we show that artemisinin kill rates may have been substantially over-estimated in previous modelling studies because (i) the parasite reduction ratio (PRR) (generally estimated as 10^4) is based on observed changes in circulating parasite number which generally over-estimates the 'true' PRR which should include both circulating and sequestered parasites, and (ii) the third dose of artemisinin at 48 hours targets exactly those stages initially hit at time zero, so it is incorrect to extrapolate the PRR measured over 48 hours to predict the impact of doses at times 48 hours and later.

Introduction

Identifying optimal deployment policies and improved drug stewardship (for example suppression of monotherapies and detection of counterfeit drugs) have become major public health objectives designed to minimise the onset of resistance of the currently recommended first-line drugs for uncomplicated malaria, i.e. the artemisinin-based combination therapies (ACTs). One method to identify best practice for their deployment is by pharmacological modelling of drug action. This has been widely used in other infectious diseases, notably bacteria (recently reviewed in (1)). Its application to malaria treatment is now being strongly recommended to optimise deployment practices (2, 3) and the World Health Organization (WHO) has recommended the development of models to improve the understanding of antimalarial drug resistance and management (4). Recent examples of pharmacological modelling can be found elsewhere (5-17), although a less mechanistic approach can also be employed by fitting curves to observed clinical data (e.g. (18)). Pharmacological models have a potentially huge impact in contributing to the rational design and deployment of drug therapies that can potentially save several million lives annually.

The conventional *in silico* method of predicting therapeutic outcome of malaria treatment is to track the number of parasites following drug treatment using ordinary differential equations (ODEs) (e.g. (19) and discussion of Equation 1 below). Some antimalarial drugs can act against liver stages and/or gametocytes but it is the asexual blood stages (rings, trophozoites, schizonts and merozoites) in human red blood cells (RBCs) that cause symptoms. In this work, we focus exclusively on modelling drug action against these asexual blood stages. This approach has one major inherent drawback when applied to malaria: it assumes the malaria parasites within a patient are entirely homogenous, i.e. that all parasites

are in identical states so that, given a certain drug concentration, all parasites are equally likely to be eliminated by the drug and, if they are not eliminated, are all equally likely to reproduce. This assumption of parasite homogeneity is violated in malaria where a single infection may harbour individual parasites that become distinctly heterogeneous as they pass through their development processes within RBCs. *Plasmodium falciparum*, the most deadly of the *Plasmodium* species causing human malaria (20), has a characteristic 48-hour infection cycle within RBCs. Parasites infect a RBC, establish several membranes and transport systems to support their subsequent development, digest and detoxify haemoglobin, and finally initiate deoxyribonucleic acid synthesis to produce the 20 to 40 new parasites that emerge from the RBC when it ruptures 48 hours after its infection. These developmental processes are reflected in large changes in the parasite metabolism. Critically, drugs are only active against those stages that utilise metabolic processes targeted by the drugs so that drug stage specificity occurs. As an example, many partner drugs in ACTs are believed to target haem digestion/detoxification and are only effective against trophozoite and schizont stages (21) when rapid haem digestion is occurring. These partner drugs, however, have long half-lives and are present at active concentrations for several 48-hour cycles after treatment so parasites pass through all stages in the presence of the drugs and the lack of stage specificity in the models is not conjectured to be too problematic. Partner drugs in ACTs are combined with artemisinins. Recent reports on artemisinin resistance potentially evolving in South East Asia lead to an increased focus on their performance (22-25). It is unknown how artemisinin resistance may affect clinical impact on therapeutic outcome and reliance on killing effects of the partner drug in ACTs is imperative. As resistance to these partner drugs starts to evolve, more pressure is placed on the artemisinin component to ensure that the ACT remains effective. Clearly, combination drugs with novel components are necessary.Artemisinins target most of the stages targeted by partner drugs (trophozoites and schizonts) but,

Antimicrobial Agents and Chemotherapy

additionally, they also act against ring stages. They also have marked differences in their potency against different asexual blood stages (see later discussion of the hyper-sensitive profile on Figure 1). The other key difference is that artemisinins have relatively short half-lives resulting in their presence at active concentration for only around 4 to 6 hours post treatment (15). Patients often present for treatment with their infections semi-synchronised around a mean developmental age of typically around 5 hours (e.g. (14)). In these circumstances, stage specificity of drug action does have an important impact: If a patient presents with parasites in stages highly sensitive to artemisinin then the drug will have a large effect. Conversely, if a patient has parasites that are predominantly in less sensitive stages, then the artemisinin drug action will be severely compromised.

Several studies have used pharmacokinetic/pharmacodynamics models that include more than one parasite stage (26-30). But to our knowledge, there has been no comprehensive evaluation of the consequences of assuming parasite homogeneity in conventional continuous-time models. Heterogeneity cannot be captured by the conventional ODE approach based on a single compartment for parasite burden in red blood cells, so the established method to investigate malaria heterogeneity and drug stage specificity is to replace the continuous-time/ODE approach with a discrete-time model using difference equations (6). This approach, first described by Hoshen *et al*. (6) and used by others (14, 15, 31), can be briefly summarised as follows: The model tracks the malaria infection by dividing the parasite development within RBC into 48 'age-bins', each bin representing 1 hour of development. These discrete-time models therefore require that each patient's treatment be described by 48 equations, each of which has to be updated for each hour of patient follow-up after treatment (typically up to 63 days (32)). While discrete-time models properly incorporate the parasite heterogeneity in malaria infections, they are computationally more

demanding. Furthermore, they have been described in principle (6) but, to date, there appears to have been no clear investigation of how they should be applied in practice for simulation of mass malaria treatment used to optimise deployment practices (e.g. alternating deployment scenarios such as age- or weight-based dosing bands or the impact of poor patient compliance in tens of thousands of malaria patients (13)).

The objectives of this study are therefore as follows. Firstly, to investigate the validity of previous models of antimalarial drug treatment that used the continuous-time approach and therefore accepted the inherent assumptions of parasite homogeneity (e.g. (5, 7-13, 18, 33)). Secondly, to quantify how much more accurate and/or less biased discrete-time approaches are and to identify their appropriate calibration from clinical, field and laboratory studies. Thirdly, to identify computational shortcuts that improve the accuracy of the continuous-time approach as the discrete-time approach is relatively slow even using modern supercomputers so that a faster continuous-time approach may provide rapid analyses appropriate in most research environments.

Methods

For clarity, the methods are presented in a qualitative, intuitive manner so that the concepts are, hopefully, accessible to non-modellers. The strategy is to compare and reconcile the continuous-time and discrete-time approaches by altering the parasite killing rates to match predicted parasite numbers between the two approaches. For simplicity we only give details on monotherapy; a discussion of how individual drug calibrations can be combined for combination therapies can be found elsewhere (12). We assume drugs may have either long

or short half-lives and either do, or do not, have stage specific killing. We look at all combinations, giving four drug types in total:

- A 'Hypothetical drug 1' with long half-life and without stage specific killing.
- An ACT 'Partner drug' with long half-life and stage specific killing. Typical examples are mefloquine and lumefantrine (killing in age-bins 18 to 40 inclusive) as well as piperaquine (killing in age-bins 12 to 36 hours inclusive) (15).
- A 'Hypothetical drug 2' with short half-life and without stage specific killing.
- An 'Artemisinin derivative' with short half-life and stage specific killing.

The two hypothetical drugs have properties that do not match any existing antimalarial drugs but are investigated for several reasons. Firstly, to understand and illustrate the general principles underlying the treatment dynamics. Secondly, novel antimalarial drugs may eventually be developed that do have these characteristics. Thirdly, the methodology is not restricted to malaria: in principle, it can be used as a general model for treatment of infectious agents with stage specificity.

The continuous-time and discrete-time approaches must be reconciled so that they yield the same observed killing rates (quantified as the parasite reduction ratio; details are in the Supplemental Material). All calculations were performed using the statistical software package R (version 3.1.1) (34).

Continuous-time models

The basic method is based on ODEs and is widely applied in simulating antimicrobial drug treatment (see (35) for a review). For malaria, an ODE is used to track the change in parasite number according to the amount of drug present, i.e.

$$\frac{dP}{dt} = P(a - f(I) - f(C))$$

Equation 1

where P is the number of parasites in the infection, t is time after treatment, a is the parasite growth rate (here we assume that each schizont releases ten merozoites that successfully re-invade RBC, giving $a = 0.048$ per 48 hours), $f(C)$ is the drug parasite killing which depends on the drug concentration C, and $f(I)$ the killing resulting from the hosts background immunity. The critical point to note is that P in Equation 1 does not distinguish between parasite developmental stages (which we term 'age-bins', see below) so this standard methodological approach cannot explicitly account for stage-specific drug action. The number of parasites at time t after treatment (P_t) is obtained using conventional calculus as

$$P_t = P_0 e^{at} e^{-\int_0^t f(C)dt}$$

Equation 2

where P_0 is the number of parasites at time of treatment, i.e. $t = 0$ (for details on how this equation is derived see, for example, the supplemental material to (11)). If the minimum predicted number is less than 1, then the infection is assumed to be cleared.

The drug killing function $f(C)$ usually follows the Michaelis-Menton equation, i.e.

$$f(C) = V_{\max}\left(\frac{(C_t)^n}{(C_t)^n + \mathrm{IC}_{50}{}^n}\right)$$

Equation 3

where C_t is the drug concentration at time t (for details see (12)), V_{max} is the maximal drug kill rate per hour or per day, IC_{50} is the concentration at which 50% of maximal killing occurs and n is the slope of the dose response curve. Two factors determine the drug killing after treatment for each drug type: its specific pharmacodynamic profile (Figure 1) and its Michalis-Menton function. The amount of drug killing plateaus at high concentrations at V_{max} (Equation 3), so a useful simplification (relaxed in Section 4 of the supplemental material) is to assume the drugs are either present and killing at maximal effect (i.e. V_{max}) or are present at negligible concentrations (i.e. essentially absent). This simple presence-absent assumption seems appropriate for the partner drugs because their long half-lives mean they are likely to be present at high concentrations over the period of the stage specific simulations, typically 4 days (= 96 hours). In the case of drugs such as artemisinins with very short half-lives, we simply define a duration of activity post-treatment (the default value being 6 hours (15)). This allows the continuous and discrete-time approaches to be matched simply by specifying a duration of time the drug is present (and killing at maximal effect) post-treatment and matching V_{max} in the continuous-time methodology (Equation 3) to its discrete-time counterpart V'_{max} (see later discussion of Equation 4): this matching will therefore enable the continuous- and discrete-time models to be directly compared.

Discrete-time models

Parasites exposed to drug treatment may be in any stage of development within their 48-hour life-cycle in RBCs and hence differ in their sensibility to the drugs. A conventional method for dealing with such continuous data is by splitting the data into a computationally-manageable number of discrete 'bins'. In principle, there can be any number and length of bins in the discrete-time model but here, following Hoshen *et al*. (6), we use a simple linear approach and split the 48-hour parasite development cycle in the RBC into 48 × 1-hour bins. We will refer to these entities as 'bins' or 'age-bins' interchangeably depending on context and need for clarity (note that Hoshen *et al*. (6) refer to them as 'boxes'). Patients may present for drug treatment with parasites in an infinite variety of distributions among these 48 bins. If drugs preferentially act against certain age-bins in the 48-hour cycle, then the distribution of parasites among the age-bins at time of treatment may have an impact on subsequent dynamics of parasite clearance. Consequently, each patient must have his/her distribution of parasites among age-bins defined at the time of treatment. For illustrative purposes, we identify five 'paradigm distributions' (PD1–5) detailed in Section 1 of the supplemental material of infections that differ in distributions at time of start of treatment. Briefly these are:

- PD1: asynchronous and equally distributed over all age-bins
- PD2: mainly in early ring stages with a relatively tight distribution across age-bins
- PD3: mainly in early ring stages with a relatively wide distribution across age-bins
- PD4: mainly in the late ring stages with a relatively tight distribution across age-bins

PD5: mainly in trophozoite stages with a relatively tight distribution across age-bins

The first step is to define a 'pharmacodynamic profile' for each drug that specifies its parasite killing for each 1-hour age-bin (Figure 1). We then combine the duration of drug killing after treatment with the drugs pharmacological profile to identify a value for the maximal drug killing rate $V'_{\max}$. These calculations are provided in Sections 2 and 3 of the supplemental material and are summarised in Table 1. The killing in each age-bin, b, at time, t, is then given as

$$V_{\max}^{b,t} = Y_b Z_t V'_{\max} \qquad \text{Equation 4}$$

where Y_b is the pharmacodynamic profile so that, in the simplest case, $Y_b = 1$ if the drug does kill parasites in age-bin b, and $Y_b = 0$ if it does not kill parasites in that age-bin. Z_t tracks the drug concentration post-treatment so that $Z_t = 1$ if the drug is present at time t, and $Z_t = 0$ if the drug is not present. This allows the proportion of parasites in age-bin b, at time t, that survive the subsequent hour to be calculated as

$$\Psi^{b,t} = e^{-V_{\max}^{b,t}} \qquad \text{Equation 5}$$

which is used in Equation 6 and Equation 7 below to track parasitaemia.

A two-dimensional matrix, the 'parasite matrix' (PM), tracks the total number of parasites in each bin for each hour post-treatment. The first column ($t = 1$) of PM holds the initial age-bin distribution of parasites at time of treatment. The algorithm then simply tracks the number of

parasites in the 48 bins after treatment using the standard index methodology dating back to Hoshen *et al*. (6) and subsequent (e.g. (14, 15, 17, 31)), i.e. for every age-bin (b) at each time (t) post-treatment, the algorithm calculates how parasites survive drug treatment and then moves the survivors on an hour into the next age-bin (i.e. b+1) and into the next time period post-treatment (i.e. t+1), i.e.

$$PM_{b+1,t+1} = PM_{b,t}\Psi^{b,t} \qquad \text{Equation 6}$$

Note that for $b = 1$ we allow for the production of new parasites at the end of age-bin 48, i.e.

$$PM_{1,t+1} = PM_{48,t}\Psi^{b,t}PMR \qquad \text{Equation 7}$$

where PMR is the parasite multiplication rate, i.e. the average number of merozoites released from a schizont that successfully infect new RBC.

Reconciling the continuous- and discrete-time approaches

The calibration requires that equivalent killing rates are identified, i.e. V_{max} in Equation 3 and V'_{max} in Equation 4, so that parasite numbers obtained from the continuous- and discrete-time methodology match at the end of each 48-hour cycle (see below). The values of V_{max} used in the continuous- and discrete-time methodologies will be distinguished by using a prime

symbol (′) for the latter, i.e. $V'_{\max}$. A hat (ˆ) above the $V_{\max}$ symbol indicates that an adjustment has been made for the effects of stage specificity and the lack of drug-killing in non-sensitive stages. A tilde (˜) above the $V_{\max}$ symbol indicates that an adjustment has been made for the short half-life of the drug and the times when the drug is absent (and hence not killing) during the 48 (or 96) hour census period.

The parasite reduction ratio (PRR) is conventionally measured in the clinic as the number of (observable) parasites present at the time of treatment divided by their number 48 hours later. The continuous- and discrete-time models can be calibrated using PRR as a metric of drug killing by making allowances for the drug's half-life and the susceptible parasite age-bins. The basic equations are given in Table 1 which shows how the kill rate calibrations depend on the amount of drug killing (i.e. PRR), the duration post-treatment that the drug is active, and parasite growth rate *a*. In the case of discrete-time modelling it also captures the number of age-bins in which killing occurs (*q*).

A problem arises with the 'Artemisinin drug' as it is impossible to match $\tilde{\hat{V}}_{\max,48}$ and $\tilde{\hat{V}}'_{\max,48}$ such that continuous- and discrete-time models give identical parasites numbers at the end of each 48-hour cycle (see later). This mismatch arises because the age-bin distribution at time of treatment has a large effect on subsequent dynamics so $\tilde{\hat{V}}_{\max}$ and $\tilde{\hat{V}}'_{\max}$ had to be matched using the parasite reduction ratio predicted to occur over 96 hours (PRR_{96}), i.e. the number of parasites present at the time of treatment divided by the number 96 hours later. The calculations required for this are given in Section 3 of the supplemental material.

Parameterisation of models

We used published results where available and attempted to identify plausible values otherwise. In all cases we use, rather than endorse these calibrations so this approach makes it straightforward for readers to calibrate the simulations according to their own local clinical and epidemiology settings.

Simulating artemisinin treatment in patient populations using continuous-time models

The methods described above allowed us to calibrate the continuous-time method such that it captures the effects of stage specificity. The obvious practical application of the new methodology is to simulate the deployment of ACTs for mass treatment of patients and to assess the impact of stage specificity on predicted population-wide drug effectiveness; the latter has been missing from previous analyses. This source of variation has not been incorporated into previous simulations of ACT treatment (e.g. (11, 12)) so we need to incorporate and assess its likely impact on the predicted treatment outcomes. We do this by re-running our previous simulations of artemether-lumefantrine (AM-LF) and artesunate-mefloquine (AS-MQ) treatment (12). The process for doing so is described in Section 3 in the supplemental material. In brief, we ran the model for multiple patients to determine the population PRR_{96} and used this to obtain a continuous-time approximation for $\tilde{\hat{V}}'_{max,96}$. This new estimate of $\tilde{\hat{V}}'_{max,96}$, and its associated inter-patient variability, was then incorporated into mass simulations of ACTs to account for the stage-specific effects of the artemisinin component.

Results

Continuous-time and discrete-time models for different types of drugs

The parasite numbers predicted by the continuous-time and discrete-time models for a drug with a long half-life that kills all parasite stages ('Hypothetical drug 1') are compared in Figure 2A. The lack of stage specific killing means that variation around the continuous-time approximation is due solely to differences caused by parasites reproducing at the end of their 48 hour cycle. Infections that were initially in late age-bins, such as PD5, will rupture and produce new parasites (merozoites) early in the 48-hour census period so parasite numbers will remain higher than the continuous-time prediction over most of the census period. Those infections that were initially in early age-bins of the cycle, such as PD2, release merozoites late in the 48-hour census period so their numbers will usually lie below the continuous-time approximation. As expected, all predicted numbers converge to the same value at the end of each 48-hour census period.

Figure 2B compares parasite numbers predicted by the continuous-time and discrete-time models for a drug with a long half-life that has stage specificity. The example shown in Figure 2B is for the 'lumefantrine' pharmacodynamic profile but similar results were obtained for the 'piperaquine' profile (Figure S3). The major difference between Figure 2A and Figure 2B is that in Figure 2B the effect of stage specificity is added to the effect of initial age-bin distributions, and variation around the continuous-time approximation is substantially increased compared to Figure 2A. The patterns of variation can be understood as

the interaction between these two effects. In an infection with parasites that are predominantly in late age-bins at the start of treatment (e.g. PD5) some parasites are killed, but many parasites do survive to rupture and release merozoites that are then unaffected by the drug for the next 18 hours (Figure 1). Consequently, parasite numbers in an infection with PD5 stay well above the continuous-time approximation for the whole census cycle. When parasites are mainly in early bins (e.g. PD2) at time of treatment, they are not affected by the drug and their total number is initially above the approximation until the time point when the parasites start to enter the sensitive bins (at 18 hours) where intense killing brings their total number down below the number predicted by the continuous-time model. Parasites initially distributed according to PD4 suffer badly from both effects as their mean age is 20.5 hours, i.e. parasites are initially killed very effectively by the drug and only when significant rupture and release of merozoites occurs around 20 hours post-treatment does their number start to re-converge towards that predicted by the continuous-time model.

Figures 2C and 2D compare parasite numbers predicted by the continuous-time and discrete-time models for a drug with a short half-life and that kills all stages (i.e. 'Hypothetical drug 2'). The major difference between Figure 2A ('Hypothetical drug 1') and Figures 2C and 2D is that 'Hypothetical drug 2' persists for only a relatively brief period after treatment. The short half-life means that such drugs would probably be given repeatedly so the dynamics are shown both for a single dose (Figure 2C) and for three repeated doses (Figure 2D). Parasite numbers initially fall rapidly and their subsequent recovery is then driven by the same dynamics as longer half-life drugs without stage specificity (Figure 2A), i.e. parasite numbers in PDs with high mean (e.g. PD5) multiply sooner in the 48-hour census period and are thus usually higher than predicted by continuous-time models, while those in PDs that have a low mean (e.g. PD2) multiply later in the 48-hour census and are thus usually lower than

Antimicrobial Agents and Chemotherapy

predicted. Critically, all PDs and the continuous-time approximation re-converge at the end of each 48-hour cycle.

Figure 3 compares the continuous-time and discrete-time models for a drug with a short half-life with the stage specific characteristics of the artemisinin class of drugs. It is extremely difficult to capture the post-treatment dynamics by a single continuous-time equation because of the impact of an infection's age-bin distribution at time of treatment. Figure 3 used the continuous-time approximation with a $\hat{\hat{V}}_{\max,\,48}$ calibrated from PD1 (using Equation S16). Note that, for instance, PD4 is very poorly captured by this approximation and, importantly, the parasite numbers do not re-converge every cycle (Figure 3A, in contrast to Figure 2A, B, C and D) so the mismatch will be perpetuated over subsequent cycles (Figure 3B). This makes it necessary to use a different continuous-time calibration for each of the five paradigm distributions by using the approach leading to Equation S26 in Section 3 of the supplemental material (Figure 4). Slight differences between the discrete- and continuous-times methods for each paradigm distribution do occur but, importantly, the continuous- and discrete-time methods always re-converge after 96 hours (Figure 4) irrespective of the age-bin distribution at time of treatment (the panels on Figure 4 illustrate five very different starting age-bin distributions) and every 48 hours thereafter as shown on Figure S4. The first convergence occurs after 96 hours because parasite killing of artemisinins has to be calibrated over a 96-hour period (rather than the 48-hour period for the other examples). The convergence in subsequent 48-hour census periods is due to the match in PMR.

Mass simulations of treatment

We replicated our recent mass simulation of AM-LF and AS-MQ treatment (12) to include stage specific drug action of artemisinins by allowing an additional two-fold variability around artemisinin $\hat{\tilde{V}}_{max,96}$ (Equation S28). Its inclusion made very little difference to the results (Figures S5 and S6 and Table S2): Cure rates using our original mean $\hat{\tilde{V}}_{max,96}$ of 27.6 per day changed from 84.74% to 84.13% for AS-MQ and from 92.29% to 91.76% for AM-LF. There was similarly a very small effect of stage specificity when we reduced artemisinin $\hat{\tilde{V}}_{max,96}$ to 14.6 per day (the reasons for using this lower artemisinin $\hat{\tilde{V}}_{max,96}$ are explained below.)

Discussion

Comparison of output from continuous-time and discrete-time models for different types of drugs

The calibrations presented in the supplemental material and summarised in Table 1 enabled the continuous- and discrete-time methods to be calibrated in an equivalent manner. This allowed us to investigate the extent to which the continuous-time approximation captures the more biologically-realistic discrete-time models.

Initial investigations used the simplest example, 'Hypothetical drug 1' which is assumed to have a long half-life and kill all age-bins. This isolated the effect of replicating at the end of the RBC life-cycle as being the only difference between the continuous- and discrete-time approaches. Results suggest that replication solely at the end of the 48-hour cycle introduced

only a small amount of variation around the treatment dynamics predicted by a continuous-time approach (Figure 2A). The discrepancy between predicted and actual numbers is small, about plus/minus half a log10 unit, and importantly is constant over subsequent cycles. The latter point is important because the infection is deemed to have been cleared if the expected number of parasites falls below 1, and variation around predicted parasite number at that point is relatively low suggesting the continuous-time approximation for therapeutic outcome (i.e. cure/fail) should be applicable for this type of drug. Our (subjective) interpretation of these results is that the assumption of continuous replication is unlikely to have a significant impact on the results from studies where drugs lack stage specific activity.

The next step was to add stage specific drug action to a long half-life drug (i.e. the ACT partner drugs). This combined the impact of stage specificity with that of replication occurring only at the end of the 48-hour life-cycle. The results are illustrated on Figure 2B. As might be expected, stage specificity introduces considerably more variation around the continuous-time approximation. These are important examples as they characterise an antimalarial 'partner' drug whose treatment has been previously examined using a continuous-time approach both by us (e.g. (11-13)) and by others (e.g. (7, 10, 33)). An important, and long overdue, question is the extent to which the continuous-time approach truly predicts the drug post-treatment parasite dynamics. We would argue, again subjectively that the approximation is good. The key factor is that the variation disappears every 48 hours and that it scales with parasite number such that maximum deviation is around two log10 units, i.e. a factor of 100. The continuous-time approach defines the infection as 'cured' when the predicted number of parasites falls below 1. Figure 2B and Figure S3 suggest this may arise if the predicted number was within two log10 units ether side, i.e. from 0.01 to 100. It seems intuitively likely that discrepancies of this relatively small magnitude would rarely

occur and, consequently, that continuous-time simulations would be accurate. This argument also assumes the worst-case scenario, i.e. that the drug instantaneously disappears at exactly the point when the discrepancy is maximal. In reality, the smooth transition from maximum killing to ineffective concentrations would likely help smooth out the discrepancies.

The third drug class investigated were drugs with a short half-life and without stage specific killing (i.e. 'Hypothetical drug 2'). The short half-life means that parasite numbers initially fall rapidly but recovered once the drug is not present anymore (Figure 2C and D). The change in parasite number is driven by the same dynamics as longer half-life drugs without stage specificity (Figure 2A) and the continuous-time approximation re-converge at the end of each 48-hour cycle. This re-convergence, plus relatively small deviations between the model types suggest that, should such an antimalarial be discovered and deployed, that the continuous-time methodology would be an appropriate simulation method.

Finally, the effects of short half-life, stage specific killing and replication only at the end of the 48-hour cycle was investigated (i.e. the artemisinin derivatives). The implications are much more serious for the continuous-time approach. Figure 3 shows the dynamics of artemisinin treatment: Deviation from the continuous-time approximation is larger, e.g. around 3 log10 units or 10^3-fold in the case of PD4 and, critically, the deviation does not periodically disappear (as it does every 48 hours for partner drugs, see Figure 2B and Figure S3). Consequently, deviations persist over time and will plausibly have an impact on predicted therapeutic outcome. In our opinion, this is an unacceptable level of divergence and we conclude that artemisinin treatment cannot be adequately modelled in the same way as the other drugs because the initial age-bin distribution at time of treatment has such a large effect on the PRR.

Figure 4 shows that a continuous-time approximation calibrated for initial bin distribution accurately tracks killing over the 2 × 48-hour parasite life-cycles that artemisinins are present, and supports our assertion that employing infection-specific continuous-time kill rates $\hat{\tilde{V}}_{\max,96}$ (Figure 4, Figures S7) can capture the variation introduced into post-treatment dynamics by patients' differing age-bin distributions at time of treatment. The essence of our argument is that the effects of differing bin distribution at time of treatment can be incorporated simply by inflating the variation in a drug's maximal kill rates.

Estimates of artemisinin kill rates

The inclusion of stage specificity into our recent mass simulation of AM-LF and AS-MQ treatment [12] made very little difference to the results (Figures S5 and S6 and Table S2). There was similarly a very small effect of stage specificity when we reduced artemisinin $\hat{\tilde{V}}_{\max,96}$ to 14.6 per day (the reasons for investigating this reduced are explained below). The analyses show that artemisinin kill rates ($\hat{\tilde{V}}_{\max,96}$ ~0.6 per hour; Table 2, Figure S7) are much lower (by a factor of around two) than estimated in our previous studies which used values of 27.6 per day (12, 13), equivalent to 1.15 per hour (i.e. 27.6/24). There appear to be two underlying reasons for this. Firstly, the use of PRR to calibrate the killing, secondly the extrapolation of PRR to overall kill rates; each will be discussed in turn.

Previous simulations of artemisinin treatment were calibrated using the observed PRR (i.e. the reduction in circulating and sequestered parasites) of around 10^4 reported in the literature

and defined as the reduction in the number of parasites observed in the peripheral blood by microscopy. This is potentially misleading because they do not capture changes in the number of sequestered parasites. Our simulations allow us to calculate both "apparent" and true" PRR and suggest that apparent PRR_{48} is substantially larger than the true PRR_{48} (Table 2). The effect of short pulses of stage specific artemisinin killing on observable, circulating parasites (age-bins up to 14) and sequestered parasites (age-bins 15 and above), and hence on observed PRR, varies greatly depending on the initial age-bin distribution of the parasites (Figure S10 and Figure S11).

The second factor behind the discrepancy in artemisinin maximal kill rates arises because, *in vivo*, the PRR is typically measured over 48 hours. This omits the impact of the final dose at time 48 and it is assumed that the results for the first two doses (which determine PRR) may be extrapolated for the third dose. However, a dose of artemisinin given 48 hours after the first dose will affect exactly the same age-bins already targeted by the first dose. Consequently, that third dose is likely to have much less impact than the first two doses. Calibration against PRR_{48} only captures the effects of the first two doses and will thus overestimate the impact of the third dose. Calibration against PRR_{96}, as done here, does incorporate the reduced impact of the third dose and so the estimated artemisinin kill rates $\hat{\tilde{V}}_{max,96}$ are further reduced.

As may be expected, this reduction in artemisinin kill rate may have a significant impact on simulated drug effectiveness. Our mass simulations based on previous work (12) show that reducing $\hat{\tilde{V}}_{max,96}$ from 27.6 to 14.4 per day (i.e. $24 \times 0.6 = 14.4$ to convert hourly to daily kill rates) roughly doubled the number of predicted treatment failures (Table S2).

Impact of stage-specificity on mass simulations of ACT treatment

Incorporating the two-fold variation caused by age-bin distributions again had a negligible effect as seen with the higher kill rate. The underlying reason appears to be that this two-fold variation adds very little to the natural variation in parasite sensitivity to the drug's $\hat{\tilde{V}}_{\max,96}$ whose coefficient of variation (CV) was assumed to be 0.3 (12) (this is shown in Figures S5 and S6). Recall we first sampled $\hat{\tilde{V}}_{\max,96}$ from a normal distribution to reflect the natural variation among parasites in their $\hat{\tilde{V}}_{\max,96}$ values; the resulting simulated distributions are shown as rows A and C on Figures S5 and S6. We then re-sampled $\hat{\tilde{V}}_{\max,96}$ from a two-fold range around this selected value to allow for differences in infections' age-bin distribution at time of treatment (*cf* Figure S7); the distribution of these re-sampled values are shown in rows B and D of Figures S5 and S6. Note, the variation increases slightly as this two-fold effect is included and that the distribution becomes slightly more right-skewed. The skew arises because the uniform distributions are scaled against the selected value of $\hat{\tilde{V}}_{\max,96}$ (Equation S28) so high values (at the right-hand side of the distribution) have higher additional variation that tends to slightly skew the distribution at this side. The important point is that the variation in $\hat{\tilde{V}}_{\max,96}$ values increases only marginally in rows A and C versus rows B and D on Figures S5 and S6. In effect, it appears that the additional variation introduced by artemisinin stage-specific killing and its short half-life is largely incorporated

into the natural background version in $\hat{\tilde{V}}_{max,96}$ so that the impact on cure rates, at least in our examples, is negligible (Table S2).

Variation in age-bin distributions at time of treatment therefore appear to have little impact in our simulations but there is no guarantee that this will be the case in all studies and it is good practice to incorporate this effect if possible. The results for SPP2 and SPP3 shown in Figure S7 suggest a general rule of thumb: In the absence of any better information, the natural variation in artemisinin kill rate $\hat{\tilde{V}}_{max,96}$ should be augmented two-fold to incorporate age-bin variation in patients at time of treatment. Our mass simulation, however, showed that adding this variability to an individual's drug killing rate, $\hat{\tilde{V}}_{max,96}$, did not affect predicted cure rates (Table S2). The natural variation around the mean of $\hat{\tilde{V}}_{max,96}$ is so large (i.e. CV = 0.3) that the distribution of patients' $\hat{\tilde{V}}_{max,96}$ barely changes when the correction for stage specificity is added (Figures S5 and S6).

Impact of adherence

The simulations assumed full patient adherence to 24-hour dosing intervals. However, in practice patients may miss a dose, delay a dose by several hours or finish treatment early. We investigated adherence in a previous publication (13) but assumed artemisinin doses were all equally effective. In reality, the impact of dose timing and the fact that the third dose of the artemisinin appears to have less impact suggests that a more nuanced approach could be used to investigate the impact of poor adherence. This could be incorporated in the same way as the effects of initial bin distribution, i.e. simulate a range of initial age-bin distributions with a

range of adherence patterns, compute PRR_{96} for each patient within the population and use this to generate the distribution of $\hat{\hat{V}}_{max,96}$ analogous to Figure S7 that also incorporates the effect of adherence patterns.

Conclusions

The potential impact of age-bin distribution on drug treatment may be obvious in retrospect. In fact, it is not a new idea but seems to have been lost in the artemisinin era (just when it was most relevant). The stage specific action of antimalarials has been investigated since the early 1980s (21, 36, 37) so it is therefore not surprising, that chronotherapy for malaria, i.e. the science of the timing of drug application so as to achieve optimal therapeutic success for the treatment of disease, is an old idea (38). Following administration of an ACT, the partner drug is present in the patient's blood at concentrations above the minimal inhibitory concentration (MIC) over several parasite life-cycles of 48 hours (39) so it is therefore unlikely that the timing of partner drug application would affect treatment outcome (Figure 2B). However, the artemisinins are present in the blood at concentrations above the MIC only during a very short period of time, i.e. 4-6 hours (15), and chronotherapeutic considerations seem justified (Figure 3). It is difficult to envisage exactly how this would be achieved in practice (it would be unethical to delay treatment) but more frequent dosing with artemisinins as occurs in the twice-per-day regimen of AM-LF, may help in this respect and deserves further investigation. As mentioned before, the WHO recently recommended the use of mathematical models on antimalarial chemotherapy for a better understanding of drug resistance and its management (40). The advantage of mathematical models is that they can

overcome some of the experimental, ethical or logistic issues associated with *in vitro* experiments or clinical trials on stage specificity of antimalarials.

The discrete-time methodology will remain the "gold-standard" simulation method but we believe the continuous-time methods will continue to be used in the foreseeable future because they offer a substantial increase in computational speed with, as we show in this manuscript, no compromise in the validity of their results. The increase in speed arises because the discrete-time models track 48 parasite developmental "bins" each of which has to be updated every hour (i.e. 24 times per day). In contrast, the continuous-time method tracks only the total number of parasites and, for most malaria drugs, is only updated daily. The ratio of computations (and hence basic speed) is therefore 1:(48 × 24), making the continuous-time approach >1,000-fold faster (with the exception of artemether-lumefantrine which is administered twice-daily, in which case the computational advantage halves to ~500-fold). Moreover, this simple calculation ignores the computational opportunity of time-saving by using calculus to project forward after the final dose in the continuous-time methods (see Appendix of (7)). In crude terms, this means the continuous method can run overnight (half day) what the discrete time method would take around a year to achieve. These simulations are highly suitable for parallel or batch processing over multiple computer cores, but no matter how many batches or cores are used, the 500–1,000× speed advantage still remains. Computational speed is important because malaria simulations have grown increasingly complex to take advantage of increased computational power, and large-scale modelling is envisaged to play a significant role in optimising malaria control and elimination programmes (3). For example, we have embedded a continuous-time methodology of drug treatment into the large-scale OpenMalaria micro-simulation of malaria epidemiology (e.g. (41, 42)). Testing various permutations of malaria epidemiology, transmission and clinical

practices typically takes 2–3 weeks to complete, so computational speed does remain a priority in such situations. Similarly, investigating the large number of different permutations of age- and weight-banding patterns under a variety of target dose ranges (in mg/kg, see (13)) is computational intensive and a 500–1,000× times increase in speed is extremely valuable in this context. What this paper has achieved is to validate a methodology, with particular relevance for artemisinins, which offers an extremely large increase in computational speed, and which confirms the validity of previous analyses published using the continuous-time approach.

This piece of work is overdue and ideally would have been performed before undertaking the mass simulations of malaria treatment that ignored stage specificity (we consider ourselves as guilty as anyone in this respect). It is interesting that the sizes of impact of the three features of stage specificity are in reverse-order of that anticipated at the start of this work. Stage specificity of artemisinin killing does inflate the variance associated with treatment but is largely lost in the context of 'natural' parasite variation in drug sensitivity (Figures S5 and S6) and had little impact on our predicted ACT effectiveness (Table S2). Stage specificity and the long half-life of partner drugs do have some impact on the minimum number of predicted parasites, and hence predicted therapeutic outcome, but the likely size of this effect seemed small and can be monitored by recording the minimum number of predicted parasites in each patient (Table S2). The largest effect arose from the combination of sequestration and a reduced impact of the third dose of artemisinin. This lead to estimated artemisinin killing being around half that obtained previously from a cruder interpretation of PRR over 48 hours (i.e. assuming that all parasites are observable) and had a large impact of predicted cure rates (Table S2). We would however stress these are initial conclusions based on a re-analysis of

some of our previous simulations of ACT treatment with the specific pharmacokinetic/pharmacodynamic calibrations described above. Our explicit objective here was to develop and present the computational techniques necessary to bring stage specificity into mass simulations of drug treatment regimens. In order to maintain a publication of manageable size, we chose not to undertake a systematic investigation of parameter space. We have attempted to be as transparent and flexible as possible so that users can easily calibrate and apply the techniques to their own particular settings and simulations. We strongly recommend that stage specificity be explicitly considered in simulations of malaria treatment and look forward to the results obtained from other studies.

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Table 1. Drug killing rates for the continuous-time and discrete-time models. These are the equations required to convert the discrete-time model to its continuous-time equivalent for a single patient, i.e. to match maximal parasite kill rate (V_{max} in Equation 3) in the instantaneous model to its equivalent V'_{max} in the discrete-time model (Equation 4), the latter being denoted by the prime (′) symbol. The hat (ˆ) or tilde (˜) above the V_{max} symbol indicate whether adjustment has been made for the effects of stage specificity and/or short half-life respectively to compensate for the lack of drug-killing in non-sensitive stages and times when the drug is not present during the 48 (or 96) hour census period.

Drug	**Half-life**	**Stage specificity**	**Continuous-time model**	**Discrete-time model**
'Hypothetical drug 1'	Long	No	$V_{max} = \frac{\ln(PRR_{48})}{48} + a$	$V'_{max} = \frac{\ln(PRR_{48})}{48} + a$
'Partner drug'	Long	Yes	$\hat{V}_{max} = \frac{\ln(PRR_{48})}{48} + a$	$\hat{V}'_{max} = \hat{V}_{max}\frac{48}{q}$
'Hypothetical drug 2'	Short	No	$\tilde{V}_{max} = \frac{\ln(PRR_{48}) + 48a}{t_a}$	$\tilde{V}'_{max} = \frac{\ln(PRR_{48}) + 48a}{t_a}$
'Artemisinin derivative' PRR_{48} calibration	Short	Yes	$\hat{\tilde{V}}_{max,48} = \frac{\ln(PRR_{48}) + 48a}{t_a}$	$\hat{\tilde{V}}'_{max,48} = \hat{\tilde{V}}_{max,48}\frac{48}{q}$
'Artemisinin derivative' PRR_{96} calibration	Short	Yes	$\hat{\tilde{V}}_{max,96} = \frac{\ln(PRR_{96}) + 96a}{3t_a}$	Obtained by iteration

798 *a*: instantaneous parasite growth rate over the 48-hour parasites red blood cell (RBC) cycle; PRR_{48}/PRR_{96}: reduction in parasite number over 48
799 or 96 hours (i.e. one or two parasite RBC cycles) following drug treatment, the value is different for each drug but identical for both models
800 when used for the same drug; *q*: number of one-hour bins during which killing occurs; t_a: duration of drug action after each dose.

Table 2. The impact of age-bin distribution at time of treatment on continuous-time artemisinin kill rates. True parasite reduction ration (PRR) is the reduction in total number of parasites and apparent PRR is the reduction in observable (i.e. non-sequestered and thus circulating) number of parasite per 48 or 96 hours. A discrete-time artemisinin kill rate ($\hat{\tilde{V}}'_{\max,48} = 1.164$) was obtained that gave an apparent parasite reduction ratio PRR_{48} of ~10^4 (actually 10,054) using the following assumptions: (i) uniform age-bin distribution, (ii) three doses of an artemisinin are given at times 0, 24 and 48 hours (although, obviously, only the first two doses contribute to the PRR_{48}) and persist for 6 hours following each dose, (iii) iso-sensitive pharmacodynamic profile (14), (iv) parasites immediately disappear from the circulation at age-bin 14 hours. See supplemental material for methodological detail and Table S1 for more results. The continuous-time equivalent artemisinin drug kill rate ($\hat{\tilde{V}}_{\max,96}$) is calculated from true PRR_{96} using Equation S26. Note that the discrete-time kill rates are identical for each row ($\hat{\tilde{V}}'_{\max,48} = 1.164$) so that the variation in continuous-time kill rate $\hat{\tilde{V}}_{\max,96}$ is caused solely by the differences in age-bin distribution at time of treatment. The dynamics of treatment are shown on Figure 4.

Distribution (mean, SD)	**True PRR_{48}**	**Apparent PRR_{48}**	**True PRR_{96}**	**Apparent PRR_{96}**	**Kill rate $\hat{\tilde{V}}_{\max,96}$**
PD1 (uniform)	541	10,054	125	14,268	0.52408
PD2 (10.5, 5)	2,032	20,024	416	34,692	0.59085
PD3 (10.5, 10)	518	11,873	112	17,533	0.51776
PD4 (20.5, 5)	324	84,293	34,822	8,770,475	0.83684
PD5 (35.5, 5)	1,889	3,069	397	3,145	0.58822

Figure 1. The pharmacodynamic profiles of antimalarial drugs used in the discrete-time methodology. The profiles describe the fraction of parasites killed per hour by the drug for each of the 48-hour age-bins (i.e. $1-\Psi^{b,t}$ from Equation 5). Calibration are based on an asynchronous, 'uniform' parasite infection which results in a $PRR_{48} = 10^3$ (lumefantrine, mefloquine and piperaquine) or $PRR_{48} = 10^4$ (artemisinins). We investigated two sensitivity profiles to artemisinins. The "iso-sensitive" profile assumes all parasite stages are equally sensitive to artemisinin: this is essentially the same profile as for partner drugs but with a wider range of stages being killed. The other "hyper-sensitive" profile assumes differential artemisinin killing between the stages. This seems intuitively plausible because drug sensitivity presumably depends on the metabolic processes taking place in each stage of development and also reflects recent findings that *P. falciparum* appears far more sensitive to artemisinins in the early ring stages than in later stages (43).

Figure 2. Changes in parasite numbers following treatment. The graph shows the number of parasites over time post treatment. Parasites present at time of treatment were distributed among age-bins according to paradigm distributions (PD) 1–5 described in Section 1 of the supplemental material. Note that the number of parasites is the true number, i.e. circulating plus sequestered, plus one (it is conventional to plot parasites + 1 when using a log scale because log(0) is undefined). **(A)** Drug with long half-life and equal killing in all age-bins (e.g. 'Hypothetical drug 1'). This was produced using the pharmacodynamic profile of 'hypothetical drug 1'. The discrete-time model used drug killing rate $V'_{max} = 0.1919$ and $Y_b = 1$ for age-bins 1 to 48 and the continuous-time model used drug killing rate $V_{max} = 0.1919$. **(B)** Drug with long half-life and stage specific killing (e.g. lumefantrine). This was produced using the pharmacodynamic profile of drug 'lumefantrine'. The discrete-time model used drug killing rate $\hat{V}'_{max} = 0.4005$, $Y_b = 1$ for age-bins 18 to 40 inclusive and $Y_b = 0$ for age-bins

0 to 17 and 41 to 48 inclusive and the continuous-time model used drug killing rate $\hat{V}_{max}$ = 0.1919. **(C)** Drug with short half-life and equal killing in all age-bins (i.e. 'Hypothetical drug 2') given as a single dose and assuming that the drug is present and acting at maximal killing for 6 hours post-treatment (15). The discrete-time model used drug killing rate $\tilde{V}'_{max}$ = 0.1919, Y_b = 1 for age-bins 1 to 48 and Z_b = 1 for the 6 hours the drug was present and the continuous-time model used drug killing rate $\tilde{V}_{max}$ = 1.919.single dose administered at time 0 hours (green arrow). **(D)** As for (C) but with three doses administered at times 0, 24 and 48 hours (green arrows).

Figure 3. Changes in parasite numbers following treatment by a drug with short half-life and stage specific killing (e.g. 'Artemisinin derivative'). This was produced using the iso-sensitive pharmacodynamic profile of the artemisinins (see Figure 1) and assuming that the drug is present and acting at maximal killing for 6 hours after each dose (15). Artemisinins are simulated as a monotherapy for clarity. They can later be combined to simulate combination therapies (12) so parasite numbers start to increase shortly after the final dose. Parasites present at time of treatment were distributed among age-bins according to paradigm distributions (PD) 1–5 described in Section 1 of the supplemental material. The continuous-time model used a single drug killing rate $\hat{\tilde{V}}'_{max,96}$ = 0.52408, i.e. the one calibrated to give a $PRR_{48} = 10^4$ for a uniform distribution (Table 2). Note that the number of parasites is the true number, i.e. circulating plus sequestered, plus one (it is conventional to plot parasites + 1 when using a log scale because log(0) is undefined). **(A)** shows the dynamics in detail up to 96 hours and **(B)** shows how the parasite numbers remain separate thereafter.

Figure 4. Changes in parasite numbers following treatment by a drug with short half-life and stage specific killing with continuous-time approximation corrected for patients' differing bin distributions at time of treatment. This was produced using the iso-sensitive pharmacodynamic profile of the artemisinins (see Figure 1) and assuming that the drug is present and acting at maximal killing for 6 hours after each dose **(15)**. Parasites present at time of treatment were distributed among age-bins according to paradigm distributions (PD) 1–5 described in the text. Unlike Figure 3 the discrete-time analysis of stage specificity and its continuous-time approximation re-converge at 96 hours for each paradigm distribution. The artemisinins have disappeared from the circulation by this time so the continuous-time approximation does capture the total amount of artemisinin drug killing. These examples use the continuous-time kill rate, $\hat{\tilde{V}}'_{max,96}$, appropriate for each distribution (Table 2), i.e. **(A)** PD1: $\hat{\tilde{V}}'_{max,96} = 0.524$; **(B)** PD2: $\hat{\tilde{V}}'_{max,96} = 0.591$; **(C)** PD3: $\hat{\tilde{V}}'_{max,96} = 0.518$; **(D)** PD4: $\hat{\tilde{V}}'_{max,96} = 0.837$; **(E)** PD5: $\hat{\tilde{V}}'_{max,96} = 0.588$. Note that the number of parasites is the true number, i.e. circulating plus sequestered, plus one (it is conventional to plot parasites + 1 when using a log scale because log(0) is undefined).