Title: Altering antimalarial drug regimens may dramatically enhance and restore drug effectiveness.

Running title: Enhancing and restoring antimalarial effectiveness

Katherine Kay (Katherine.Kay@LSTMed.ac.uk)
Eva Maria Hodel (EvaMaria.Hodel@LSTMed.ac.uk)
Ian M Hastings (Ian.Hastings@LSTMed.ac.uk)

Authors’ affiliation: Parasitology Department, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom

Corresponding author: Katherine Kay, Parasitology Department, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom. 0151 705 254 Katherine.Kay@LSTMed.ac.uk

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Abstract

There is considerable concern that malaria parasites are starting to evolve resistance to the current generation of antimalarial drugs, the artemisinin-based combination therapies (ACTs). We use pharmacological modelling to investigate changes in ACT effectiveness likely to occur if current regimens are extended from three to five days or, alternatively, given twice-daily over three days. We show that the pharmacology of artemisinins allows both regimen changes to substantially increase artemisinin kill rate. Malaria infections rarely contain more than $10^{12}$ parasites while standard dosing regimens allow approximately one in $10^{10}$ parasites to survive artemisinin treatment. Parasite survival falls dramatically to around one in $10^{17}$ parasites if the dose is extended or split; theoretically this increase in drug killing appears more than sufficient to restore failing ACT efficacy. One of the most widely used dosing regimens, artemether-lumefantrine, already successfully employs a twice-daily dosing regimen and we argue that twice-daily dosing should be incorporated into all ACT regimen design considerations as a simple and effective way of ensuring their continued long-term effectiveness.
Introduction

Artemisinin-based combinations therapies (ACTs) are the global first-line treatments for the most serious of the human malaria species, Plasmodium falciparum. Recent reports that artemisinin resistance may be evolving in South East Asia has caused considerable alarm over our ability to both treat and control this lethal infection (1-4). Artemisinin resistance alone has little clinical impact provided its partner drug within the ACT remains effective, but as resistance to these partner drugs starts to evolve, more pressure is placed on the artemisinin component to ensure that the ACT remains effective. One option to restore treatment efficacy is to replace a failing ACT with one based on a different, effective partner drug. However, the development of new partner drugs and their implementation as first-line therapy is a long and expensive process (5). An attractive alternative is to improve the stewardship of existing ACTs and to restore or maintain their clinical effectiveness through improvements to their deployment regimens.

One obvious regimen change is to simply increase the total dose given to patients as has historically been applied to several antimalarials drugs (e.g. chloroquine (6)). However, this approach means patients potentially receive dosages exceeding the target range recommended by the World Health Organization (WHO), thereby raising concerns over drug safety and potential toxicity. An alternate strategy is to maintain the same daily dose but extend the regimen e.g. from three to five days. As with increasing the daily dose, extended regimens may lead to drug safety concerns if the drug is known to accumulate in the physiological compartment where it elicits adverse effects. However, some antimalarial drugs, such as piperaquine (PPQ), are rapidly distributed away from the central (adverse effect) compartment thus reducing its accumulation and hence potential toxicity. These
prolonged treatments have been considered, for example, to overcome multi-drug resistance in malaria in the Greater Mekong sub-region (1). A final, potentially highly effective strategy has emerged from our recent investigations of antimalarial pharmacokinetic (PK) / pharmacodynamic (PD) models (7-9) and from a patient-based pharmacological analysis (10). It involves maintaining the standard three-day regimen but administering the drugs as twice-daily dosing. This strategy of twice daily dosing was shown to be effective in vitro following artemisinin treatment (11) and is already used for the ACT artemether-lumefantrine (AL) (12, 13).

These strategies are applicable to all ACTs but here we examine the specific case of dihydroartemisinin (DHA)-PPQ, a widely used fixed-dose ACT, currently given as three consecutive daily doses for the treatment of uncomplicated *P. falciparum* malaria. We selected this as a ‘model drug’ because several recent analyses suggest an increased risk of treatment failure with the current DHA-PPQ regimen, e.g. a population PK study (14), an observational cohort study (15), a pooled meta-analysis investigating over 7,000 patients (16) and predictions from a pharmacological model (9). The choice of DHA-PPQ also allowed us to use a range of one-, two- and three-compartmental PK calibrations that cover the PK characteristics of most current antimalarial drugs. We calibrate parasite resistance levels to generate drug failures rates ranging from 2-35%. These are higher than those currently being observed in the field but it is important to emphasise that DHA-PPQ is used here as a theoretical example as our approach is most useful in modelling a future scenario where drug failure rates have reached significant levels, and when a drug policy change is needed.

This study used a PK/PD simulation approach to quantify and compare the abilities of four alternative regimens to restore the clinical effectiveness of DHA-PPQ in the face of potential
drug resistance. Details of the dosing regimens are summarised in Table 1. We investigated the standard dosing regimen, i.e.

- **SR3/3**: the current standard regimen (SR) of DHA-PPQ, i.e. given as three daily doses (note the subscript ‘3/3’ indicates 3 doses over 3 days).

Two extended (prefix ‘e’) five-day regimens, i.e.

- **eSTD5/5**: the extended regimen with the same total dose (STD) as the standard regimen. Consequently the amount of drug given per day was reduced to \( \frac{3}{5} = 60\% \) that of the standard regimen.

- **eITD5/5**: the extended regimen with increased total dose (ITD). The same daily dose is given as in the current standard regimen (i.e. in SR3/3) so increasing the duration to five daily doses resulted in a \( \frac{5}{3} = 1.67 \)-fold (or 67%) increase in total dose.

Two three-day split (prefix ‘s’) dose regimens where six doses are equally spread over the three days at 12-hour intervals, i.e.

- **sSTD6/3**: the split, twice-daily regimen using the same total dose as the standard regimen. Each twice-daily dose therefore contains half the daily drug dosage of the standard regimen.

- **sITD6/3**: the split, twice-daily regimen with increased total dose. Each twice-daily dose is the same as the daily dose in the current regimen (i.e. SR3/3) resulting in patients receiving twice the total dosage given in the current standard regimen.

Current antimalarial regimens for uncomplicated malaria last a maximum of three days (17) and it is generally believed that adherence will fall if the regimens last longer than 7 days (17). Adherence is a complex topic (see later discussion) so, for illustration, we examined a
simple scenario where patients take only three of the recommend five doses in the extended
regimens:

- pa[eSTD5/5]: the scenario where poorly-adherent (prefix ‘pa’) patients take only three
  of the recommended five doses of the eSTD5/5 regimen. This regimen has reduced
daily doses of DHA-PPQ (see above) so these patients consequently receive only 3/5
of the current recommended total.

Poor adherence to eITD5/5, defined as patients taking only three of the required five doses, is
equivalent to patients taking the standard three-day regimen (see description of eITD5/5
above); hence pa[eITD5/5] ≡ SR3/3.

Methods

Pharmacokinetic/pharmacodynamic model
Drug treatment was simulated using a PK/PD mechanism-based modelling methodology as
described in previous publications on malaria drug treatment (7-9, 18, 19). The model tracked
parasites numbers as a function of parasite growth and changing drug concentration. The PK
parameters do not alter in the simulations (for example, there are no dosage-induced changes
in elimination rate).

Pharmacodynamic component
The PD component of the model, i.e. *P. falciparum* sensitivity to DHA and PPQ, followed
Michaelis-Menten kinetics and was modelled using the calibrations described previously (7)
and validated against field observations. For DHA, the mean slope factor (n) was 4 with a
 coefficient of variation (CV) of 0.3, the mean \( IC_{50} \) was 0.009 mg/L (CV = 1.17) and the mean
kill rate (\( V_{\text{max}} \)) was 27.6 per day or 1.15 per hour (CV = 0.30). For PPQ, \( n = 6 \) (CV = 0.30),
\( IC_{50} = 0.088 \) mg/L (CV = 0.30) and \( V_{\text{max}} = 3.45 \) per day (CV = 0.30). Note that the \( IC_{50} \) for
PPQ lies towards the upper range of reported values and its implications for failure rates for
simulations based on two- and three-compartment PK models are discussed above.

Pharmacokinetic component for dihydroartemisinin

The PK component for DHA was also calibrated and validated previously (7). The mean
central volume of distribution (\( V_{dc} \)) was 1.49 L/kg (CV = 0.48) and mean elimination rate
constant (\( k \)) was 19.8 per day (CV = 0.23), the latter being equivalent to a half-life of
\[
\ln(2)/19.8 = 0.035 \text{ days or 0.85 hours.}
\]

Pharmacokinetic component for piperaquine

There are several published estimates of PPQ PK parameters but the structural models differ
in the number of compartments required to describe the PK profile, some using two
compartments (20-22) and some using three compartments (14, 23, 24). This study
concentrated on the results of a previously calibrated and validated one-compartment model
(7) plus three calibrations of the two-compartment PK models (20-22) and three calibrations
of the three-compartment PK models (14, 23, 24). Details are as follows.

One-compartment model for piperaquine

The one-compartment PK model for PPQ used \( V_{dc} = 150 \) L/kg (CV = 0.42) (25) and \( k = 0.03 \)
per day (CV = 0.54) (21). Using the methods described in Winter & Hastings (7), this
calibration (‘calibration 1’) predicted a maximal drug concentration (\( C_{\text{max}} \)) of 0.12 ng/mL.
following a 18 mg/kg single PPQ dose which was within the range reported by Chinh et al. (25).

Two-compartment models for piperaquine

The methodology required to simulate a two-compartment PK model, assuming first-order absorption, linear elimination and multiple doses (without lag time) was described previously in the Appendix of Kay & Hastings (8) and in Bertrand & Mentré (26). The two-compartment PK model was initially calibrated using data from a recently published PK study with DHA-PPQ in Cambodia (20) ('calibration 2a') and then with published PK studies from Thai patients (22) ('calibration 2b') and Cambodian adults (21) ('calibration 2c'). The mean PK parameters, their distributions, the typical patient body weight and the original dosing regimen (used in the PK studies) are given in Table 2. To validate each calibration, the predicted $C_{\text{max}}$ and time to $C_{\text{max}}$ ($t_{\text{max}}$) were simulated and compared with the observed values from the original publications as follows: calibration 2a, Figure 6 and raw data from (20); calibration 2b, Figure 5 from (22); calibration 2c, Figure 3 from (21).

Three-compartment models for piperaquine

The methodology required to simulate a three-compartment PK model with first-order absorption, linear elimination and multiple doses (without lag time) was described by Bertrand & Mentré (Equation 1.72 in (26)). The three-compartment PK model was calibrated using data from three recently published PK studies of DHA-PPQ in Thai pregnant and non-pregnant women (23) ('calibration 3a'), Burkinabe children (14) ('calibration 3b') and Sudanese pregnant and non-pregnant women (24) ('calibration 3c'). The mean PK parameters, their distributions, the typical patient body weight and the original dosing regimen (used in the PK studies) are given in Table 2. To validate each calibration, the
predicted $C_{\text{max}}$ and $t_{\text{max}}$ were simulated and compared with the observed values from the original publications as follows: calibration 3a, Figure 3a from (23); calibration 3b, Figure 3 from (14); calibration 3c, Figure 6 from (24).

Ratio of dihydroartemisinin to piperaquine in the simulations
An important operational point, specific to DHA-PPQ, is that the WHO currently recommends dosing with a DHA to PPQ ratio of 1:4.5 (17). This ratio means patients treated with the co-formulated drug combination get the appropriate amount of each drug. However, for historical reasons, the commercially available co-formulations routinely used as first-line therapies in malaria-endemic countries contain a DHA to PPQ ratio of 1:8 (27). The component with the narrower therapeutic window, in this case PPQ, dictates the upper dosage of the combination that can be given to patients, so treatment using these co-formulations systematically gives patients less than the recommend 4 mg/kg dosage of DHA. Both the recommended ratio and the actual co-formulated drug ratio were checked for putative regimen changes.

Simulations
The PK/PD models were each used to simulate 5,000 infected patients, followed for 63 days after treatment to determine the ‘true’ cure rate, ‘apparent’ cure rate and parasite clearance time (PCT) of each regimen (Table S1). The true cure rate was defined as the proportion of patients whose infections were completely cleared. The apparent or observed cure rate was defined as the proportion of patients whose infections were below the microscopic limit of detection, defined as $10^8$ parasites, on days 28, 42 and 63 post treatment. The apparent cure rate is the most likely measure of effectiveness to be reported from clinical trials. The PCT is the time taken for a patient’s infection to drop to undetectable levels, again defined as $<10^8$.
parasites. Individual PK/PD parameters were sampled from a normal distribution for parameters when the coefficient of variation (CV) was less than 50% and from a log-normal distribution when CV ≥ 50% (see (8) for details of the latter). Each patient was simulated six times to receive each of the five dosing regimens and the poor-adherence scenario. Note, we do not consider transmission intensity as we assumed all individuals in the simulation to be infected with a single malaria clone. This provides the treatment outcome ‘per clone’ and avoids the more complicated analyses required when simulating multi-clonal infections (see (28) for more information). Details of the dosing regimens are given in Table 1 and were either based on the target dose recommended by the WHO, where the DHA to PPQ ratio is 4:18 = 1:4.5 (17), or based on the commercially available co-formulation where the DHA to PPQ ratio is 1:8 (27) (dosing regimens in Table S2 and results in Table S3 and Figure S1).

Results

A one-compartment model clearly fails to capture the concentration-over-time profile of PPQ but it does reproduce observed clinical failure rates (Figure 2 of Hodel et al. (9) compared to Table 6 and Figure 4 in the WorldWide Antimalarial Resistance Network study (16)). This one-compartment model was useful in developing the methodological underpinning for antimalarial PK/PD modelling and, although pharmacologically rather crude, it did allow the reliable replication of observed field and clinical data (e.g. Winter & Hastings (7)). A wider range of failures rates were predicted for two- and three-compartment models using the standard regimen (the SR3/3 columns in Figure 1) and are a consequence of using an half-maximal inhibitory concentration (IC50) for PPQ at the upper range of observed values (29). Estimates of IC50 values vary considerably between studies and whilst the in vitro measures
of IC50 may not necessarily represent its in vivo value, an IC50 of 0.088 mg/L was used previously (7) as an appropriate calibration for a one-compartment model. PPQ concentrations decline much faster in the more realistic two- or three-compartment models so PPQ IC50 should, in principle, be reduced to allow more killing in the shorter time period when the drug is present in the central compartment where killing occurs. We chose to retain the IC50 of 0.088 mg/L in these two- and three-compartment models as this is a theoretical example that can be most usefully applied to future situations in which clinical efficacy has declined. It was also deemed preferable to maintain the high IC50 value to simulate the situation where drug resistance had started to evolve and where cure rates were declining (30, 31). The current standard regimen, SR3/3, gave cure rates of 65-96% for two- and three-compartment models (Table S1), which spans the threshold of 90%, the point at which WHO state the drug should be replaced (32). This range of cure rates were ideal to achieve the primary, key objective of this study, i.e. to test whether regimen changes gave consistent patterns of results over a range of calibrations and basal drug failure rates.

The predicted cure rates and parasite clearance times (PCTs) for all seven PK/PD calibrations and all dosing regimens are given on Table S1. Figure 1 illustrates the predicted cure rates for the regimens based on the WHO recommended target dose ratio of 1:4.5 DHA to PPQ (i.e. 4 mg/kg/day DHA and 18 mg/kg/day PPQ (17).

The cure rates of the novel regimens (assuming patients are fully adherent) were very high (>97%, Table S1) for the one-compartment calibration (7) but much more variable for the more biologically realistic two- and three-compartment models. Extending the regimens from the standard three-day regimen (SR3/3) to either of the five-day regimens (eSTD5/5 and eITD5/5) reduced estimates of failure rates in the model across all calibrations. This was true
even when failure rates were particularly low; for example, the 2% failure rate for SR3/3 in calibration 1 was cut to 0.6% (eSTD5/5) and 0.3% (eITD5/5) representing at least a three-fold reduction in failure rate. Both the split, twice-daily dosing regimens (sSTD6/3 and sITD6/3) also dramatically reduced treatment failure rates (Figure 1). Notably, the sITD6/3 gave the highest cure rates for all simulated regimens (92-99.9% cure rates across all calibrations, dark blue columns in Figure 1) while the sSTD6/3 consistently performed better than the five-day eSTD5/5 (grey vs. green columns in Figure 1) and had cure rates broadly similar to those of the eITD5/5 regimen (grey vs. pale blue columns in Figure 1). These results were obtained using the WHO’s recommended ‘optimal’ DHA:PPQ ratio; repeating these results using the DHA:PPQ ratio of the commercially available co-formulation gave qualitatively near-identical results (Table S2 and Figure S1). The quantitative differences were a relative reduction in cure rates of 1-14% when compared with the equivalent result using WHO ratio (note this is a relative reduction, i.e. 80% cure rate reduced to 60% represents an absolute reduction of 20% but a relative reduction of 25%).

Poor adherence to the five day regimen was defined here as missing the last two doses and dramatically reduced treatment cure rates. In the case of poor adherence to eITD5/5, cure rates were, by definition, those of the SR3/3 (pale blue vs. red columns in Figure 1). Poor adherence to eSTD5/5 gave the model output presented in pa[eSTD5/5] (green vs. orange columns in Figure 1), i.e. cure rates were reduced by approximately 7% in calibration 1, 35-50% in calibration 2 and 8-38% in calibration 3 (again note this is a relative reduction, see above).

The variation in $C_{\text{max}}$ associated with changing the recommended SR3/3 regimen to eITD5/5, sSTD6/3 or sITD6/3 was estimated for the two- and three-compartment model calibrations and shown in Figure 2. The median $C_{\text{max}}$ increase occurring in the simulated eITD5/5 regimen lies...
in the range of 13-63% above that of the standard regimen (eITD_{5/5}: SR_{3/3} in Figure 2). The median $C_{\text{max}}$ increase occurring in the simulated sITD_{6/3} regimen lies in the range of 75-99% above that of the standard regimen (sITD_{6/3}: SR_{3/3} in Figure 2). As expected, splitting the total dose over 6 doses did reduce median $C_{\text{max}}$, the decrease was between 1-12% (sSTD_{6/3}: SR_{3/3} in Figure 2) depending on the PK model structure and the model calibration. Figure 2 summarises the model output from individual simulated patients and it is important to note the considerable inter-patient variability around these median changes in $C_{\text{max}}$ (see later discussion).

Discussion

A fundamental medical principle is that the physician should “above all, do no harm” (33). Consequently, we first discuss the implications of the regimen changes for drug safety, before turning to our primary concern of how they change drug efficacy. Adverse drug effects may be driven by different mechanisms depending on the drug. The principle safety concerns relate to the maximal drug concentration occurring in a patient after treatment (i.e. $C_{\text{max}}$), total drug exposure (quantified as the area under the concentration-over-time curve) or an average drug concentration over a certain time period after treatment. In the specific example of PPQ, safety concerns relate mainly to $C_{\text{max}}$ so the changes in $C_{\text{max}}$ were estimated for the two- and three-compartment calibrations and shown in Figure 2. Splitting the current three-day regimen (SR_{3/3}) into twice-daily dosing (sSTD_{6/3}), maintains the same total dose so Figure 2 shows the expected result: that a patient’s $C_{\text{max}}$ will be no higher and probably much lower, than if they took the current standard regimen. In contrast, extending the standard regimen SR_{3/3} over five days (eITD_{5/5}) does increase total dose so will inevitably increase PPQ $C_{\text{max}}$ by a factor of up to $5/3 = 1.67$. Similarly, giving the standard dose twice-daily
(sITD6/3) doubles the total amount of PPQ given with a consequent increase in patients’ $C_{\text{max}}$. Importantly, Figure 2 reveals considerable inter-patient variability around these changes in median $C_{\text{max}}$. This variability arises because PPQ is distributed away from the central compartment into one or more peripheral compartments. Patients who slowly transfer PPQ from the central to the peripheral compartment(s) will tend to accumulate PPQ in their central compartment over subsequent doses and will have higher $C_{\text{max}}$ ratios. Conversely, patients with a high transfer rate will accumulate less PPQ between doses so will have much lower $C_{\text{max}}$ ratios. The differences in median $C_{\text{max}}$ observed in the different calibrations may be explained in the same way, i.e. calibrations where transfer from the central compartment is relatively slow tend to accumulate PPQ and have higher median values for $C_{\text{max}}$ changes. As a caveat, it is impossible to estimate a definitive value for the changes in $C_{\text{max}}$ associated with regimen changes without applying more sophisticated absorption/distribution models to the raw PK data although we believe our estimated changes in $C_{\text{max}}$ are qualitatively fairly accurate because any PK approximation(s) will apply to all doses given in the regimen.

In the specific case of DHA-PPQ, safety concerns focus on the PPQ $C_{\text{max}}$ values which are associated with QTc prolongation (heart rhythm corrected QT interval; a prolongation of the time between the start of the Q wave and the end of the T wave in the heartbeat cycle). This occurs in a dose-dependent manner determined by the $C_{\text{max}}$ in the central compartment (for recent data see (34, 35)). There is currently no consensus on how to translate PPQ $C_{\text{max}}$ to a risk of QTc prolongation but presenting the likely size and variability of increases in $C_{\text{max}}$ associated with changes in regimen (Figure 2) serves two purposes. Firstly, it proves a point of principle that changes in $C_{\text{max}}$ do not scale with changes in total dose. For example total PPQ intake increased by a factor of $5/3=1.67$ in eITD5/5 but $C_{\text{max}}$ increased by a median factor of around 1.1 to 1.5 depending on the PK calibration (Figure 2) and this point needs to be...
recognised when regimen changes are being evaluated in terms of potential safety. Secondly, these distributions of $C_{\text{max}}$ provide a resource to interpret any future estimations of the relationship between $C_{\text{max}}$ and QTc. The important policy implications of these results are that (i) proposals to extend the duration of DHA-PPQ regimens will not drive an increase in median $C_{\text{max}}$ proportional to the changes in total dosage, but that (ii) there will be considerable inter-patient variation around this median so that extended regimens will almost certainly elicit significant $C_{\text{max}}$ increases in a minority of patients.

These concerns over safety need to be balanced against changes in drug effectiveness. The key result is that changing the current standard, once-per-day three-day regimen to any of the proposed alternate regimens predicted dramatically improve drug cure rates (Figure 1, Table S1) and that these simulated results were consistent across all seven PK calibrations and for treatment with drugs containing either the WHO or commercially-available DHA:PPQ ratios (Supplemental Information). Even in calibration 1 where true cure rates were already high (>97%), changing to five-day regimens was predicted to reduced failure rates at least a three-fold. As might be expected, treatment with the optimal WHO recommended ratio of DHA:PPQ improved simulated cure rates by up to 14% when compared to the equivalent commercial co-formulated regimen containing a lower proportion of DHA. For example, calibration 2b predicted cure rates of 67.5% (Table S1) when patients were given the current standard regimen (SR3:3) conforming to the WHO predicted DHA:PPQ ratio and 58.1% when the ratio of DHA:PPQ was reduced to match that of the commercially available co-formulation (Table S2), hence the 14% relative reduction.

The four proposed regimen changes all greatly increased cure rates but each had practical advantages and disadvantages. The eSTD5:5 regimen was effective without drastic increases
in $C_{\text{max}}$, but our simulations suggest poor adherence has the potential to substantially reduce
the efficacy of this regimen; Figure 1 shows the drop in cure rates that would occur if only
three of the five daily doses were taken (i.e. comparing the green eSTD5/5 column with the
orange pa[eSTD5/5] column). The sITD6/3 regimen raises safety concerns: the total dose is
doubled and the $C_{\text{max}}$ is increased considerably in all parameterisations (Figure 2). We
believe these practical drawbacks are sufficiently serious to preclude the use of these two
regimens as first-line therapies in most clinical situations although readers are entitled to
make up their own minds. The remaining two regimens, eITD5/5 and sSTD6/3, appear more
plausible as first-line therapies. The extended eITD5/5 regimen has two main potential
drawbacks: the possible impact of poor adherence to a five-day regimen (i.e. comparing the
pale blue eITD5/5 column with the red SR3/3 column in Figure 1) and the 67% increase in total
dose and the subsequent increases in $C_{\text{max}}$ (shown in Figure 2). In contrast, the sSTD6/3
regimen is the least critical in terms of safety concerns: total dose stays the same so PPQ $C_{\text{max}}$
is reduced (Figure 2). Its only potential drawback may be poorer adherence with a twice-daily
regimen. A systematic investigation of the impact of poor adherence would be a complex task
(36, 37). There are various patterns of non-adherence depending on whether doses are missed
and/or delayed, and operationally this all occurs in a context where people are dosed
according to age- or weight bands which further inflates the variation in drugs dosage
actually taken by patients. An example of the required approach can be found in (9) but it is a
complex analysis and this study focuses on a more specific research question, i.e. the extent
to which modifying regimens can help offset the evolution of resistance. A clear next step
would be to utilise the predictions made herein to inform the design of empirical studies from
which more specific recommendations can be derived. One ‘problem’ at present is that all
ACTs appear to be currently highly effective (but see (15)) and it is extremely difficult to
statistically identify a regimen that improves cure rate from, for example, 96% to 98% (even
although failure rate has halved from 4% to 2%). Our objective has therefore been two-fold. Firstly to identify robust regimens that are theoretically capable of delaying and offsetting the threats posed by drug resistance while, secondly, establishing a pharmacological framework to explore dosing options to be explored if or when drug failure rates start to rise to unacceptable levels.

There is a precedent for the split-dose strategy for malaria treatment. AL is possibly the most widely used antimalarial drug and is given as a twice-per day regimen for three days. This regimen was designed because lumefantrine absorption from the gut saturates at high drug levels so a strategy of giving smaller amounts of lumefantrine more frequently increases the total amount of drug absorbed (38), hence the choice of twice-daily dosing. Interestingly, adherence to this twice-daily regimen appears not to be markedly reduced (36). The presence of an existing twice-daily antimalarial regimen would also help the deployment of new twice-daily regimens of other antimalarial drugs. Most clinics and private health-providers stock a range of antimalarial drugs and poor adherence can arise because of confusion among patients or health-providers about exactly how a regimen is to be taken (39). Consistency between the various ACTs regimens (i.e. if all antimalarials were to be taken twice daily over three days) may therefore help reduce confusion and improve overall adherence to all antimalarials.

It is relatively easy to explain why increasing the duration of treatment or splitting a three-day regimen with daily doses into a three-day regimen with twice-daily dosing dramatically increases overall ACT effectiveness. This arises because treatment with artemisinins follows the law-of-diminishing returns identified and discussed briefly by us (Figure 5 of (40)). This effect is illustrated in Figure 3. Panel (a) shows DHA concentrations after treatment with...
either the standard 4 mg/kg dose given in the current standard regimen or a single 2 mg/kg
dose given when this standard once-daily dose is split to twice-daily. The law of diminishing
returns arises because these concentrations on Panel (a) are converted into parasite killing
through the Michaelis-Menton function shown on Panel (b). Killing saturates at higher drug
concentrations so the higher concentrations achieved by the standard dose in Panel (a) are
superfluous for most of the time post-treatment. This effect is shown in Panel (c) which
shows killing rates post treatment. As can be seen, halving the dose has only a small impact
on total killing measured as the area under the kill curve. This effect can be quantified
‘intuitively’ by noting that the standard dose persists at active killing concentrations for
around 7 hours post-treatment (Panel (c)). Doubling the dose would, by definition shift this
curve to the right by one DHA half-life, i.e. by 0.85 hours. This would translate into an
increase in total killing by a factor of around \((7+0.85)/7 = 1.12\) and this 12% increase in total
killing is much less than the factor of two by which dosage was increased. More relevant to
our regimens is the halving of the dose: this shifts the kill curve to the left resulting in a
decrease in total killing of around \((7-0.85)/7 = 6.15/7 = 0.88\) and again this 12% reduction in
killing is much less than the two fold-reduction of drug used. The 2 mg/kg dose is given
twice per day so the increase in killing compared to a standard 4 mg/kg single daily dose is
by a factor of \(2 \times 0.88 = 1.76\) or by 76% per day. More precise calculations can be made by
obtaining the area under the drug kill curve (Panel (c)) by simple integration. The values are
7.94 kill days for the standard dose and 6.98 kill days for the split-dose. Splitting the three
daily 4 mg/kg doses into six twice-daily 2 mg/kg doses dramatically improves total DHA
killing because, critically, these measurements are on an exponential scale (i.e. the areas
under the drug kill curve are the integration terms in the Appendix of Simpson et al. (18) and
in equation (16) in Kay & Hastings (8)). The total amount of parasite killing by DHA in a
three-day standard ACT regimen is therefore \(\exp(3 \times 7.94) = 2.2 \times 10^{10}\) and in the three-day
split dose regimen is \( \exp(6 \times 6.98) = 1.54 \times 10^{18} \). The same effect explains the increased
efficacy of the five day extended dosing regimen whose DHA killing can be calculated as
\( \exp(5 \times 7.94) = 1.7 \times 10^{17} \). Technically, these drug kill rates are incorporated into PK/PD
modelling by using their reciprocals to predict parasite survival (hence the negative signs
associated with the integration terms in the Appendix of Simpson et al. (18) and in equation
(16) in Kay & Hastings (8)). In plain English, this states that one parasite in \( 2.2 \times 10^{10} \) is
predicted to survive DHA treatment in the standard regimen and approximately one in \( 1.54 \times 10^{18} \) or one in \( 1.7 \times 10^{17} \) for the split and extended regimens respectively.

This is a remarkable result as it suggests that the simple expedient of splitting the DHA dose
can increase its overall killing within an ACT by a factor of \( 10^8 \), an increase that appears
easily sufficient to restore efficacy to failing ACTs. These results are consistent with early
clinical trials showing three-day treatments with artemisinin monotherapies had high failure
rates and that regimens required five to seven daily doses to be effective (41). These
observations can therefore now be easily explained by noting that a symptomatic malaria
infection typically contains \( 10^{10} \) to \( 10^{12} \) parasites. The three-day standard regimen is
estimated (see above) to have the potential to kill approximately \( 10^{10} \) of the parasites so is
unlikely to clear all parasites in the infection. Conversely the split regimens and extended
five-day regimens have the potential to kill approximately \( 10^{18} \) and \( 10^{17} \) parasites respectively
and so should reliably clear most infections. These are ‘average’ kill rates and the large
variability associated with DHA PK/PD (see methods) introduces substantial variation into
the DHA killing rates and explains why split or extended regimens do not cure all infections.
In addition, the stage specificity of artemisinin, i.e. the extent of drug action depends on the
exact stage of parasites in their intra-erythrocytic life cycle, also introduces variation into the
overall kill rates although it is much less than that introduced by PK/PD variation. This
increased killing is also less likely to allow spontaneous new resistance mutations to survive treatment and enter the parasite population, thereby extending the ACT’s therapeutic lifespan (42).

These results for extended and split regimens will also apply to ACTs containing other artemisinin derivatives such as artesunate and artemether. These compounds are rapidly converted into their active metabolite DHA so the PK are functionally very similar. The results are also generalizable to all the current partner drugs. We focussed on DHA-PPQ as a specific example because it is the ACT considered most at risk of currently failing, and also because it allowed us to investigate the consistency of PPQ results across one-, two-, and three-compartmental PK models. All partner drugs are believed to follow one of these structural models so the consistency of results across models and calibration suggests the results are robust (and can be easily tested for individual partner drugs by different calibrations of the methodology described herein). Note, as explained above, that regimen changes improve therapeutic outcome primarily by increasing artemisinin killing. The total killing by partner drugs is relatively unaffected by its exact dosing schedule during the first three to five days they typically have much longer half-lives.

These types of PK/PD analyses are valuable precursors to, but cannot replace, the clinical trials that serve as the gold standard upon which policy decisions will be made. However, clinical trials on drug effectiveness are time consuming, expensive, and ethically cannot knowingly administer sub-therapeutic treatment regimens to patients in order to quantify the impact of poor adherence. Moreover, if the original regimen is still largely effective, clinical trials cannot realistically detect the increased underlying killing in the alternative regimen as cure rates in both treatment arms will be high and failures often attributable to factors...
common to both arms, such as poor adherence, sub-optimal drug metabolism or re-infections. As an example of this, Das et al (43) did investigate a split-dose regimen but cure rates were high in both arms and no differences in parasite clearance rates (used as a proxy for drug effectiveness) were observed. This probably arose because parasite clearance rates are affected by a huge number of other, non-drug factors, particularly patient immunity so that, as we show in a companion paper (44), even very large changes in drug effectiveness will be essentially invisible against the dominant role of host immunity on determining parasite clearance rates. Further discussion on the usefulness of clearance rates as proxy for drug effectiveness can also be found in References (44-46). This modelling methodology represents a fast and inexpensive approach to inform policy makers. Moreover, the consensus methodologies are published, allowing easy replication of the results when alternative calibrations are provided, and allowing further research into operational questions such as their optimal dosing bands (9). In particular splitting the current ACT regimens into twice daily dosing comes close to being the ‘holy grail’ of regimen changes: it maintains the current three day regimens, raises no additional safety concerns, increases artemisinin kill rates by a huge margin, and appears easily capable of restoring the effectiveness of failing ACTs. Moreover the underlying reasons for these properties are easily understood intuitively and are applicable to the whole range of current ACTs although, importantly, the benefits are largely restricted to the artemisinin component and cannot alter the threat posed by evolution of resistance to the partner drugs (e.g. (47)). We believe that changes in ACT regimens should be instigated as soon as possible to mitigate and/or avert the spread of resistance and that this approach seems greatly preferable to the more traditional one of first allowing resistance to arise and then attempting to find methods of overcoming it.
Acknowledgements

We thank Pascal Ringwald for encouraging us to investigate extended five-day regimens of DHA-PPQ and Ghaith Aljayoussi for access to unpublished work on $C_{\text{max}}$ increases associated with different structural PK models. This work was supported by the United Kingdom Department for International Development, the World Health Organization, the Bill and Melinda Gates Foundation [grant number 37999.01 to IMH via the Swiss Tropical and Public Health Institute]; and the Medical Research Council [grant number G1100522 to IMH]. The policy implications discussed in the manuscript reflect our own beliefs and should not be interpreted as necessarily reflecting those of our funders.
References


formulations of dihydroartemisinin and piperaquine in Vietnamese subjects.


44. Hastings IM, Kay K, Hodel EM. Submitted. How robust are malaria parasite clearance rates as indicators of drug effectiveness and resistance?


Figure 1. The percentage of individuals predicted to be cured by five dihydroartemisinin-piperaquine (DHA-PPQ) dosing regimens and one poor adherence scenario. Cure rates were estimated across seven different pharmacokinetic (PK) calibrations for PPQ. The regimens and poor adherence scenario are described in the main text and summarised in Table 1. The PK calibrations are as follows (see main text for more details).

Calibration 1 is the one-compartment PK model described in 7. Calibrations 2a-c are two-compartment models calibrated using data from (20-22) respectively. Calibration 3a-c are three-compartment models calibrated using data from (14, 23, 24) respectively.

Figure 2. The changes in piperaquine (PPQ) maximal drug concentration ($C_{\text{max}}$) that are predicted to occur as regimens are either extended from three to five days or given twice daily for three days. The changes are plotted as the ratio between the $C_{\text{max}}$ predicted for the new regimen compared to $C_{\text{max}}$ predicted in the same patient following the current standard regimen (i.e. SR3/3). The red dashed reference line shows a ratio of one, which indicates no change in the $C_{\text{max}}$ associated with a regimen change, and the box plots show the ratios obtained for 5,000 individual patients. The regimens are detailed on Table 1 and explained in the main text but, briefly, the left panel shows the change associated with extending the current regimen from three to five days. The central panel shows the change associated with splitting the current dose into twice daily dosing and maintaining the same total dose. The right panel shows the change associated with giving the current dose twice-daily which obviously results in patients being given twice the existing dosage. The $C_{\text{max}}$ changes were estimated across six PK calibrations as described in the main text and in the caption to Figure 1.
Figure 3. Why splitting dihydroartemisinin (DHA) doses has such a large impact on overall killing. (a) DHA drug concentrations following dosing with either the standard daily dose (4 mg/kg) or half that amount as used in the split doses, i.e. 2 mg/kg. (b) How DHA concentration translates into parasite killing (per hour) using the standard Michaelis-Menton kill curves (e.g. Equation 1 of (8)) and the DHA parameters given in the main text. (c) Post-treatment drug killing (per hour) by DHA, obtained by converting the concentration profiles of DHA in (a) into drug kill rates using the function in (b).
Table 1. Dosing regimens, and a poor adherence scenario, for dihydroartemisinin-piperaquine (DHA-PPQ) investigated in this study.

<table>
<thead>
<tr>
<th>Regimen*</th>
<th>Dosing interval</th>
<th>Single dose DHA/PPQ [mg/kg]</th>
<th>Total dose DHA/PPQ [mg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR3/3 (Standard regimen)</td>
<td>Once daily for three days</td>
<td>4/18</td>
<td>12/54</td>
</tr>
<tr>
<td>eSTD5/5 (Extended regimen)</td>
<td>Once daily for five days</td>
<td>2.4/10.8</td>
<td>12/54</td>
</tr>
<tr>
<td>pa[eSTD5/5] (eSTD5/5 with poor adherence)</td>
<td>Once daily for three days (doses four and five missed)</td>
<td>2.4/10.8</td>
<td>7.2/32.4</td>
</tr>
<tr>
<td>eITD5/5 (Increased and extended regimen)</td>
<td>Once daily for five days</td>
<td>4/18</td>
<td>20/90</td>
</tr>
<tr>
<td>sSTD6/3 (Split SR3/3)</td>
<td>Twice daily for three days</td>
<td>2/9</td>
<td>12/54</td>
</tr>
<tr>
<td>sITD6/3 (Twice SR3/3)</td>
<td>Twice daily for three days</td>
<td>4/18</td>
<td>24/108</td>
</tr>
</tbody>
</table>

* The ratio of DHA-PPQ in the combination is 1:4.5 and corresponds to the ratio of the target dose recommended by the World Health Organization. The dosing regimens containing the commercially available fix-dose combination (1:8 ratio) can be found in Table S2.
Table 2. Mean pharmacokinetic parameters of piperaquine (PPQ) for the two- and three-compartment model calibrations.

<table>
<thead>
<tr>
<th>Study population</th>
<th>Cambodian adults and children (n = 60)</th>
<th>Burmese and Karen adults (n = 87) and children (n = 11)</th>
<th>Cambodian adults (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW</td>
<td>42</td>
<td>48</td>
<td>47</td>
</tr>
<tr>
<td>D</td>
<td>2.9/22.9 at 0 and 24 h, 1.9/15.2 at 48 h</td>
<td>7/55 split in three or four doses</td>
<td>1.7/13.6 at 0, 6, 24 and 32 h</td>
</tr>
<tr>
<td>( CL )</td>
<td>( 108 \times BW^{0.75} ) [1.01]</td>
<td>( 1584 \times (1 + 0.0262 \times (BW - 48)) ) [0.42]</td>
<td>–</td>
</tr>
<tr>
<td>( k_{e} )</td>
<td>11.2 * [2.17]</td>
<td>17.2</td>
<td>1.99</td>
</tr>
<tr>
<td>k</td>
<td>–</td>
<td>–</td>
<td>1.4</td>
</tr>
<tr>
<td>( Q_{1} )</td>
<td>69.7 [1.01]</td>
<td>65.5</td>
<td>–</td>
</tr>
<tr>
<td>( k_{12} )</td>
<td>–</td>
<td>–</td>
<td>4.6 [0.57]</td>
</tr>
<tr>
<td>( k_{21} )</td>
<td>–</td>
<td>–</td>
<td>0.1 [1.31]</td>
</tr>
<tr>
<td>( V_{d1} )</td>
<td>346.0 [0.93]</td>
<td>8,660 \times (1 + 0.0273 \times (BW - 48)) / BW [1.01]</td>
<td>14.5 [0.46]</td>
</tr>
<tr>
<td>( V_{d,p1} )</td>
<td>443.0 [1.70]</td>
<td>500.0</td>
<td>559.5b</td>
</tr>
</tbody>
</table>

Continued on following page
Table 2. – Continued

<table>
<thead>
<tr>
<th>Study population</th>
<th>Calibration 3a (14)</th>
<th>Calibration 3b (14)</th>
<th>Calibration 3c (24)</th>
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<tbody>
<tr>
<td></td>
<td>Thai pregnant (n = 24) and non-pregnant (n = 24) women</td>
<td>Burkinabe children (n = 236)</td>
<td>Sudanese pregnant (n = 24) and non-pregnant (n = 24) women</td>
</tr>
<tr>
<td>BW</td>
<td>48</td>
<td>18</td>
<td>53</td>
</tr>
<tr>
<td>D</td>
<td>2.1/17 at 0, 24 and 48 h</td>
<td>12.4/8.8 at 0, 24 and 48 h</td>
<td>2.3/18.1 at 0, 6, 24 and 32 h</td>
</tr>
<tr>
<td>CL</td>
<td>939.1 ± 0.71</td>
<td>10.0 ± 0.38</td>
<td>12.1 ± 0.45</td>
</tr>
<tr>
<td>k_a</td>
<td>3.5 ± 1.08</td>
<td>1.99 ± 1.08</td>
<td>1.99 ± 1.08</td>
</tr>
<tr>
<td>Q_1</td>
<td>106.8 ± 1.02</td>
<td>17.5 ± 5.32</td>
<td>10.8 ± 0.94</td>
</tr>
<tr>
<td>Q_2</td>
<td>52.0 ± 0.70</td>
<td>14.4 ± 0.66</td>
<td>119.5 ± 0.51</td>
</tr>
<tr>
<td>V_d</td>
<td>57.6 ± 0.86</td>
<td>13.7 ± 2.16</td>
<td>34.3 ± 0.53</td>
</tr>
<tr>
<td>V_d_p1</td>
<td>92.5 ± 1.24</td>
<td>14.1 ± 2.15</td>
<td>300.0 ± 0.60</td>
</tr>
<tr>
<td>V_d_p2</td>
<td>654.2 ± 0.65</td>
<td>185.6 ± 0.65</td>
<td>141.9 ± 0.51</td>
</tr>
</tbody>
</table>

n: number of patients; BW: body weight [kg] of the typical patient in the original study; D: Dose of dihydroartemisinin and piperaquine [mg/kg] administered to the typical patient in the original study; CL: clearance [L/day]; k_a: absorption rate per day; k_e: elimination rate per day (k_e = CL/V_d); Q_1: inter-compartmental clearance [L/day/kg]; Q_2: inter-compartmental clearance [L/day/kg]; V_d: central volume of distribution [L/kg]; V_d_p1: volume of distribution [L/kg] in peripheral compartment 1; V_d_p2: volume of distribution [L/kg] in peripheral compartment 2. The coefficient of variation (CV) around the parameter value is given in square brackets. a The parameter was reduced by 50% compared to the original study (20). b Calculated from the steady-state volume of distribution (V_dss), i.e. V_d = V_dss - V_d, and reported only for completeness (V_d is not required for calculations and the CV was not given in the original study). c CL, Q_1 and Q_2 were reduced by 35%, 50%, 10% and 10% respectively compared to the original study. d Value taken from Hung et al. (21) and increased by 75% compared to the original study. e Value taken from Hung et al. (21). f CL, Q_1 and Q_2 were reduced by 40%, 50% and 25% respectively compared to the original study.