The pharmacokinetic determinants of the window of selection
for antimalarial drug resistance

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Abstract
The selection and spread of antimalarial drug resistance poses an enormous challenge to the health of people living in tropical countries. Most antimalarial drugs are slowly eliminated and so, following treatment in endemic areas, provide a gradient of concentrations to which newly acquired parasites are exposed. There is a variable period during which a new blood stage infection with resistant malaria parasites can emerge from the liver and subsequently produce gametocyte densities sufficient for transmission, while reinfection by sensitive parasites is still suppressed. This “window of selection” drives the spread of resistance. We have examined the factors which determine the duration of this window, and thus the resistance selection pressure. The duration ranges from zero to several months and is dependent on the degree of parasite resistance, the slope of the concentration-effect relationship, and the elimination kinetics of the antimalarial drug. The time at which the window opens and the duration of opening are both linear functions of the terminal elimination half-life. Because of competition from sibling susceptible parasites, the greater risks of extinction with low starting numbers, and opening only when blood concentrations have fallen below the minimum inhibitory concentration, the window of selection for de-novo resistance is narrower than for resistance acquired from elsewhere. The window was examined for currently available antimalarials. Drugs such as the artemisinins and quinine with elimination half-lives of less than one day do not select during the elimination phase.

Introduction
The development of antimalarial drug resistance can be divided into two discrete parts; first the relatively rare de-novo emergence event, and then the survival, multiplication, and subsequent spread to other humans of the resistant parasite progeny of that event. Resistance can emerge de-novo within an acute infection, when a spontaneously arising drug resistant mutant malaria parasite is selected by antimalarial concentrations which are sufficient to eliminate the susceptible parasite population, but still allow survival and subsequent multiplication of the mutant (23). As parasite numbers are highest in the acute infection this event is most likely to happen at the time of the mitotic division which preceded the peak parasite density. In a proportion of cases (determined by the
multiplication rate) the mutation will have happened in an earlier division (i.e. before drug treatment) and several resistant parasites will be present. Selection of resistant parasites occurs if antimalarial concentrations eradicate the drug sensitive parasites but do not cause extinction of the mutant subpopulation. Selection only leads to spread if the mutant resistant parasite population expands, produces gametocytes, and these are transmitted (13).

Spread of resistance also depends on this selection process; resistant parasites acquired from elsewhere (i.e. from another person carrying resistant parasites, or much more rarely a de-novo event in a mosquito) can survive and multiply in the presence of residual antimalarial drug concentrations which inhibit the multiplication of drug sensitive parasites. Watkins and Msobo (20) highlighted the importance of antimalarial pharmacokinetics, notably the drug’s terminal elimination half-life, in determining the selection pressure driving the spread of resistance. As high grade resistance to antimalarial drugs is usually a stepwise process, and rarely occurs with a single genetic event (single point mutations in cyt b conferring atovaquone resistance are the exception to this), de-novo selection is more likely to occur when a large infecting parasite population is exposed to sub-therapeutic concentrations of a single antimalarial drug (12, 23). The resistant parasite or parasites will survive and multiply when the concentrations of drug in the blood of the patient are below the level required to keep the multiplication rate of the resistant subpopulation of parasites below 1, or in other words when concentrations are below the minimum inhibitory concentrations for the resistant parasites [MIC\textsubscript{R}]. The resistant population will then re-expand, as the drug is eliminated and concentrations fall further, eventually causing a recrudescence of the infection and, critically, producing gametocytes for transmission (1,22). There has been considerable debate as to the relative importance of the primary infection versus exposure of newly acquired infections to residual drug levels as the source of de-novo resistance to antimalarial drugs, and the degree of selection provided by slowly eliminated antimalarials. Rapidly eliminated antimalarials are thought to provide little or no selection opportunity during the elimination phase.
The aim of this paper is to investigate the relationship between the pharmacokinetic characteristics of the antimalarial drugs, the resistance attributes (pharmacodynamics), and parasite multiplication rates to determine the drug specific opportunities for the selection of resistance during the elimination phase.

**Methods and Assumptions**

For antimalarial resistance to spread resistant parasites must be transmitted. If a resistant parasite occurs de-novo during an acute infection then its progeny must multiply sufficiently to generate transmissible gametocyte densities. Selection refers to the survival advantage in the presence of antimalarial drug conferred by genetic changes in the malaria parasite. We concentrate on pharmacological aspects and do not deal with other important factors determining the spread of resistance such as fitness and recombination breakdown of multigenic resistance. Immunity, which is such an important determinant of malaria population dynamics, is discussed only in relation to selection.

Sporozoite inocula are skew distributed with median values of 6-10, but on occasions up to 100 may be inoculated (14, 16). Each successfully infected hepatocyte will produce $10^4$ to $10^5$ merozoites - each of which can invade a red blood cell (6). If a malaria infection emerges from the liver while there are concentrations of antimalarial sufficient to kill both sensitive and resistant parasites then there can be no selection of resistance. Thus between 10,000 to 1,000,000 parasites emerge from the liver to start the asexual infection of red blood cells (6).

If the infection emerges from the liver when antimalarial blood concentrations have fallen to levels which will not drive resistant parasites to extinction, but will extinguish a sensitive infection, then there is the opportunity for selection. For resistant parasites acquired from elsewhere, all or, in a multiclonal infection, many of the parasites emerging from the liver will be resistant. Thus for a drug with only blood stage activity the “window of selection” opens immediately after emergence of parasites from the liver. At this stage the antimalarial drug concentrations may temporarily inhibit parasite multiplication, (i.e. exceed the MIC$_R$) but selection is possible as long as these
concentrations do not drive the new resistant parasite population to extinction (Figure 1a). The window of selection closes when antimalarial blood concentrations have fallen to levels such that the survival probabilities of resistant and sensitive parasites drug concentrations are equal.

In the rare event that a resistant parasite emerges de-novo during intrahepatic development, or in one or more of the merozoites emerging from the liver, then the dynamics are slightly different (Figure 1b). This usually single parasite’s progeny must survive (and stochastic effects mean that not all would survive even in the absence of drugs) and then outstrip the growth of approximately 100,000 of its sensitive sibling parasites emerging from the liver to generate sufficient gametocytes for transmission. Thus for the progeny of a single resistant mutant to survive the parasite multiplication rate must exceed 1, that is the drug concentrations must be < MIC$_R$ (22). So for a de-novo resistant parasite’s progeny to survive the level of resistance needs to be higher than for resistant parasite acquired from elsewhere. The single or relatively few de-novo resistant mutants are then in a “race” with their otherwise identical drug sensitive siblings to attain parasite densities sufficient to transmit. This numerical advantage of the sensitive sibling parasites balances the “start” provided by the greater multiplication of the resistant parasites in the race to produce gametocytes, and so creates a boundary condition for elimination half-life, relative to the degree of resistance induced below which de-novo selection during the elimination phase cannot take place (22). This boundary condition is parameterised by the slope and the right shift in the concentration effect-relationship (i.e. the degree of resistance). Asynchronous hepatic schizogony further tips the balance in favour of the majority sensitive parasites because in most cases schizonts containing the sensitive parasites will have liberated merozoites before the schizont bearing the resistant parasite ruptures.

**Mathematical model**

We assume that the concentration of antimalarial drug is at the maximum at time = 0, then drug is eliminated in a first order process so that

$$C(t) = C(0) * e^{-kt}$$

(1)
where $k = \ln(2) / t_{1/2}$, $k$ is the terminal elimination rate constant, $t$ is time in days and $C(t)$ is concentration at time $t$, $C(0)$ is the maximum concentration. Although many antimalarial drugs have a more complex elimination profile, selective concentrations are present mainly or only during the terminal elimination phase following correct dosing.

Therefore we assume for simplicity that de-novo selection events or infection with a resistant parasite take place during the terminal elimination phase, although multiphasic elimination could be incorporated by using a series of exponential terms. The pharmacodynamic effects of the antimalarial drug are characterized by parasite killing, a first order process of fractional reduction in numbers per asexual cycle (4), which can be considered as the reciprocal of parasite multiplication (22).

The range of multiplication causing parasite expansion has a lower bound of just over one, and an upper bound provided by the mean number of viable merozoites per schizonts (approximately 34). Failure of merozoites to invade reduces this but cases of highly efficient multiplication have been documented (PMR>20). In these examples the parasite multiplication rate (PMR) when there is no drug effect, is assumed for simplicity to equal 10 (i.e. PMR =10), which corresponds approximately to values obtained in early volunteer studies (5,18). Although at higher densities parasite expansion slows and eventually stabilizes, the volunteer data indicate exponential growth in the density range examined here.

The maximum effect of the drug is a PMR= $10^{-3}$. This corresponds to parasite killing by drugs such as quinine, chloroquine or mefloquine against sensitive parasites (22). Parasite killing is synonymous with reduction in parasite numbers in the blood, and is a first order process (4).

The relationship between the parasite killing and drug concentration $C$ (concentration-effect or dose-response relationship) can be described by a sigmoid Emax model:

$$f(C) = - k_1 \cdot \frac{C^n}{(C^n + EC50^n)}$$

where $k_1$ is the first order rate constant of maximum parasite killing; in this case
\[
\ln(10^{-3})/2, \ n \text{ is the slope the concentration-effect curve, } C \text{ is the antimalarial drug concentration, and } EC_{50} \text{ is the drug concentration which produces 50% of the parasite killing achieved at maximum effect (Emax).}
\]

Further, we assume for simplicity that the shape of the concentration-effect relationship is the same in the susceptible and resistant populations (i.e. the same slope \( n \) and the same maximum effect) but that the curve is parallel-shifted to the right in the resistant population, and the degree of shift is reflected by the ratio of respective \( EC_{50} \)'s:

\[ p = \frac{EC_{50} \text{ (resistant)}}{EC_{50} \text{ (sensitive)}.} \] ................................. (3)

Although a parallel shift is examined initially for the purposes of illustration more complex changes in the shape of the concentration-effect relationship with resistance could be incorporated.

The mathematical relationship between the total parasite burden and time, in the presence of drug, was described by Simpson (17):

\[
P(t) = P(0) \cdot e^{k_2 t} \cdot \left( \frac{EC_{50}^n + C(0)^n e^{-nkt}}{EC_{50}^n + C(0)^n} \right)^{-k_1 / (k_1n)} \] ...............(4)

where \( P(t) \) is total number of parasites in the blood or the total parasite biomass at time \( t>0, \) and \( P(0) \) is the total number of parasites at time \( t=0, \) \( k_2 = \ln(10)/2 \) is first order rate constant of parasite multiplication rate (per day), \( C(0), EC_{50}, \) \( k, k_1 \) and \( n \) are defined as before.

In studying treatment effects on the acute infection the parasite biomass usually exceeds \( 10^8 \) parasites in an adult (and correspondingly less in a child). This is the lowest value for the pyrogenic density, and thus a threshold at which for the patient feels ill and seeks treatment (11). In intermittent presumptive treatment the parasite biomass is generally lower, as the drug recipients are usually well (25).
In studying selection after resolution of the acute infection when reinfections emerge from the liver in the presence of residual drug levels, lower numbers of parasites ($10^4$ to $10^5$) are present (6).

So if the maximum drug concentration $C(0)$ is reached at time $t=0$, and the blood stage infection starts later i.e. at time $t = t_0 > 0$ with $P_s(t_0)$ sensitive parasites and $P_r(t_0)$ resistant parasites, then at time $t > t_0 > 0$ we will have $P_s(t)$ sensitive parasites and $P_r(t)$ resistant parasites as shown:

$$P_s(t) = P_s(t_0) \cdot e^{k_2(t-t_0)} \cdot \left( \frac{1+a^n e^{-nkt}}{1+a^n} \right)^{-k_1 l(kn)} \quad \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots \cdots 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2. the ratio of the maximum drug concentration (at time 0), C(0) and EC50 - \( a \).
3. the slope of the linear portion of the concentration-effect relationship – \( n \).
   We have assumed that the slope of this relationship is the same in-vitro as in-vivo (15) even if the intercept terms differ.
4. the ratio of EC50’s for resistant and sensitive parasites \( p \)

**Resistant infection characteristics**

Two scenarios were examined. In the first resistance arises as a new event among the newly acquired parasite population. In the second resistant parasites are acquired from elsewhere.

**Scenario A: De-novo genetic event conferring antimalarial resistance (Figure 1 b)**

We assume that one resistant parasite occurs de-novo at time \( t_0 \), during the infection with sensitive parasites in the presence of residual antimalarial drug.

\[ PM(t_0) = P(t_0) \cdot 10^x \]

\( PM(t_0) \) is the probability of a resistant parasite occurring in an individual at time \( t_0 \). The de novo mutation event probability depends on the total number of parasites in the body at time \( t_0 \), \( P(t_0) \), and the mutation rate \( 10^x \); \( x \leq 0 \) (23).

\[ PI(t_0) = PM(t_0) \cdot PS(t_0) \]

\( PI(t_0) \) is the probability of developing a resistant infection, \( PI(t_0) = PM(t_0) \cdot PS(t_0) \), where \( PS(t_0) \) is the probability of the resistant mutant surviving.

We will assume that the resistant infection cannot be transmitted until the resistant parasite subpopulation forms \( \geq 10\% \) of the overall parasite population and there are about \( 10^8 \) parasites in total, that is when the resistant population can produce enough gametocytes to have a reasonable chance of transmitting (i.e. \( 10^8 / 10 = 10^7 \)) (6, 11).

**Scenario B: Infection with a resistant parasite (Figure 1a)**

We assume that following antimalarial treatment the patient acquires a new resistant malaria infection from elsewhere and at time \( t_0 \) approximately 100,000 resistant parasites emerge from the liver. We will assume that the resistant infection reaches transmissible
densities when the population in the blood reaches $10^7$ parasites in total. That is when there could be enough gametocytes of the resistant population produced to be ingested in a normal 2-3uL mosquito blood meal and form a zygote in the anopheline vector (6). This is a rather generous threshold; it assumes complete conversion of asexual to sexual stages. The transmission potential of *P. falciparum* is low at these densities, and increases until gametocyte densities reach 1000/uL (1,11), whereas *P. vivax* is more efficiently transmitted at low densities.

We call the time interval when the drug concentrations allow the resistant infection to develop but not the drug sensitive infection a *window of selection*.

**Window of selection**

**Scenario A: De-novo genetic event conferring antimalarial drug resistance**

The opening point of the window of selection is defined as the first time $t_0$ when the resistant parasite can survive; that is when the PMR for resistant parasites is unity (or $dP/dt = 0$) and afterwards grows steadily to reach at least 10% of the subsequent total parasite burden (figure 1b). The threshold of 10% is an arbitrary point in a continuous distribution describing the relationship between the proportion of resistant asexual parasites, the resulting generation of gametocytes bearing the resistance genetic mutations or gene duplications, and the consequent probability of transmission.

The closing point of the window of selection is defined as time after which concentrations of the drug are so low that there is no selective pressure for resistance. In other words the difference in PMR for sensitive and resistant parasites is such that the earlier selected resistant parasite numbers will not reach 10% of the total parasite burden.

**Scenario B: Infection with a resistant parasite from elsewhere**

The opening point of the window of selection is defined as the first time $t_0$ when the drug effect in subsequent cycles will not kill all the resistant parasites in the body and so the resistant parasite population can survive and later expand after time $t_1$.

The closing point of window of selection is the time point at which there is no preferential survival of the resistant parasites.
These points were estimated for a number of different pharmacokinetic parameters in a simulation study.

**Simulation study**

In the simulation study the important variables affecting resistance selection probability in newly emergent infections arising after an antimalarial treatment were varied. Table 1 lists values of pharmacokinetic parameters (a, n, t$_{1/2}$, p) which were investigated. In Table 4 we show the pharmacological characteristics of current antimalarial drugs for comparison. The objective was to characterize the conditions required for potential preferential transmission of resistant parasites (i.e. selection of resistance). For each set of the pharmacokinetic parameters, emerging resistant and sensitive parasite populations were simulated starting at time 0 (when the drug is at the maximum concentration) and then at 1 hour intervals up to the closing point of the selection window.

For each given set of values of a, n, t$_{1/2}$ and p, and t$_0$, the expanding populations of resistant and sensitive parasites (or resistant parasites only) were simulated using formulas (1) and (2) until it was clear whether or not the resistant population could survive and attain the required proportion for transmission (at least 10% of the total infecting parasite population) when the total parasite burden reached $10^8$. The maximum value of t$_0$ for which the resistant population could still reach the required size was taken as the closing point for the selection window.

Linear regression was used to characterize the relationship between drug half-life (t$_{1/2}$) and the opening and the closing points for the window of selection. The minimum antimalarial drug elimination half-life for which a selection window exists was estimated as a half-life for which the opening and the closing times were the same, in other words when regression lines fitted to the opening points and the closing points cross. If this point corresponded to a negative value, then the minimum half-life was estimated as the value for which the closing point’s regression line crosses the x-axis. In order to obtain the precise estimates, the regression lines were fitted to the opening and
closing times only for half-lives less than 4 days as very slight curvature was observed for some combinations of parameters ((p=4,a=5,n=5) and (p=4,a=5,n=10)).

As antimalarial drugs show stage specificity in their action (19), and infections may be synchronous the minimum half life for which the selection window is at least 12 h (24h) wide was estimated.

**Sensitivity analysis**

Sensitivity analysis was performed for the number of sensitive parasites emerging from the liver, parasite multiplication rate, and the maximum drug effect. All simulations for window A and resistance intensity p=2 were repeated for (a) $10^6$ and $10^7$ sensitive parasites emerging from the liver at time $t_0$; (b) parasite multiplication rate of 2 and 5; (c) maximum parasite killing rate $k_1$ of $\log(0.01)/2$ and $\log(0.1)/2$.

All simulations were run in Java\textsuperscript{TM} and results were analysed using Stata 9.0 (Stata Corp. 2005. Stata Statistical Software: Release 9. College Station, TX: Stata Corp LP.)

**Results**

Two scenarios were examined; A: resistance arising in a newly acquired infection and B: acquisition of a resistant infection from elsewhere.

**Scenario A: De-novo genetic event conferring antimalarial drug resistance**

Calculated windows of selection for different parameters are shown in Figure 2. Importantly a window of selection does not exist for all drug half-lives. Rapidly eliminated drugs provide no selective pressure in many circumstances. High initial drug concentrations (a=100), high levels of resistance, and a steep dose-response relationship lower the half-life limit for “no selection”. Table 2 gives the shortest drug half-lives for which the window of selection exists. In Table 3 the shortest half-lives for which the selection window is at least 12h and 24h wide (i.e. a quarter or half of the single asexual cycle) are given. In the examples given there is no selection provided by half-lives of less than two days. Except for extreme levels of resistance there is no selection with
elimination half-lives of less than one day, and for very rapidly eliminated drugs such as
the artemisinin derivatives ($t_{1/2} \leq 1$ hour) there is no window under any condition.

For small ratios of C0/EC50, any slope of the concentration/effect curve, and an intensity
of resistance represented by parameter $p$ greater than 2, the window of selection opens
very early, even at $t=0$. There are small differences (up to 30%) between the duration of
the window of selection for different pharmacodynamic parameters at the small ($p=2$) and
medium ($p=4$) intensities of resistance. The differences are more profound only between
different levels of ratios of C0/EC50 with the highest intensity of resistance ($p=6$), where
window width increases by up to 50% for the medium ratio and up to 100% for the
highest ratio of C0/EC50.

The relationship between opening point, closing point, and width of the selection
window, for a given resistance intensity, the slope of the concentration/effect curve, and
the ratio C0/EC50, are all well estimated by a linear function of the half-life of the drug
with positive slopes (all R2>0.99 except for two cases for the opening point:
($p=4,a=5,n=5$) and ($p=4,a=5,n=10$) when R2>0.96). As expected for comparable levels of
antimalarial activity, opening and closing of the window occurs later for drugs with long
half-lives than for drugs with short half-lives. The width of the selection window is
directly proportional to the length of the terminal half-life. The ratio of the duration of the
selection window to the elimination half-life is equal to a median (range) value of
1.11 (1.01- 1.3) for resistance intensity ($p$) of 2, 2.10 (1.66-2.30) for resistance
intensity of 4, 2.60 (1.66-2.89) for resistance intensity of 6, across all values of the other
parameters used in the simulation. It should be noted that drugs with very long terminal
half-lives such as chloroquine and piperaquine have a multiexponential decline in plasma
concentrations, and therefore selection for higher levels of resistance may occur in the
distribution phase. This effectively shortens the window of selection for increasing levels
of resistance.

Slopes of the linear relationships between opening and closing points and the duration of
the window depend on other pharmacodynamic parameters i.e. the slope of the
concentration/effect curve and the ratio C0/EC50 and also on the intensity of the resistance.
For the window opening point, the slope is inversely correlated with the slope of the concentration/effect curve, but directly proportional to the C0/EC50 ratio, and decreases with increasing intensity of resistance.

For the window closing point, the effect of the pharmacodynamic parameters was similar but there were no differences between different resistance intensities. Consequently, the width of the window of selection increases with increasing drug resistance since the window opens sooner for higher levels of resistance but the closing points are the same for different intensities of resistance. The selection window for higher intensity resistance contains the selection window for the lower intensity.

Table 4 gives the estimated selection windows for current antimalarial drugs. For drugs with short half-lives - artemisinin and quinine - the window of selection effectively does not exist for resistance intensities \((p) \leq 20\).

**Sensitivity analysis**

In the sensitivity analysis the opening of the window remained the same for \(10^5, 10^6\) or \(10^7\) parasites emerging from the liver (), but the window closed earlier for the higher numbers of sensitive parasites. This resulted in differences in window durations of about 1 day for a slope of the concentration-effect of 3 and about 0.6 days for a slope of the concentration-effect of 10, for each 10-fold increase in the number of emerging parasites. Decreases in the maximum parasite killing rate increased values of half-life for which the selection window exists and created wider windows. The minimum half-life for the selection window to exist was approximately 2 days for a parasite reduction ratio (PRR) of 1000 (rate = \(\log(0.001)/2\)) (22); approximately 4 days for a PRR of 100 and around 8 days for a PRR of 102. For longer half-lives the increases in the window duration were constant between PRRs of 1000 and 100 for each combination of parameters, and varied between 2.5 and 3.5 days for different values of \(n\). The window was the widest for a weak antimalarial effect (PRR = 10) and, when it existed, always opened at time of the parasite emergence from the liver. For this rate, the increase in window duration was much steeper than for higher rates.
Correspondingly the duration of window decreased for lower parasite multiplication rates; the window opened later but the closing times were the same for all rates. The reduction was up to 70% for a half-life of 5 days and up to 50% for a half-life of 20 days.

**Scenario B: Infection with a resistant parasite acquired from elsewhere**

Figure 3 shows window opening times for each half-life and for each combination of the pharmacokinetic parameters and resistance. If the resistant infection starts (i.e. emerges from the liver) earlier than these times, the resistant infection will be killed by the antimalarial drug levels, if the resistant infection starts later than these times, but before “window closure” it will develop and will have a chance to be transmitted.

For low initial drug concentrations ($C_0/EC_{50} = 3$ or 5) and for short drug half-lives, the infection will never be eradicated so the window opens at time $t=0$.

There is a linear relationship between the opening point and the drug half-life, for any given resistance intensity, slope of the concentration/effect curve, and the ratio $C_0/EC_{50}$. This relationship always has a positive slope i.e. opening of the widow occurs later for drugs with longer half-lives (Figure 3). The slope increases with increasing antimalarial concentrations at time 0. The slope of the concentration/effect curve had relatively little effect on the opening times for the values studied. The selection window obviously opens earlier if the level of resistance is higher. The time when the window closes is proportional to the half-life, it increases with “a” and decreases with “n”. The resistance level does not have any effect on the time of window closure. Figure 4 compares the selection window for scenario A, and opening and closing times for scenario B on the same graph. Figure 5 shows the relationship between the width of windows for scenario A and B and Table 4 gives the selection windows for current antimalarials. This table provides a representative selection of drug susceptibilities as examples. A window exists for all drugs, and has a very long duration relative to the drug half-life if the ratios of $C_{\text{max}}$ to the IC50 are large.

The principal conclusions from these simulation exercises are that the terminal elimination half–life is the main determinant of the time of onset and duration of the
window of selection for both scenarios. The maximum drug concentrations (Cmax) do affect the duration of the window only for high levels of resistance, for lower levels of resistance Cmax only changes time at which the window opens. Steeper slopes in the concentration-effect relationship (higher value of n) slightly (in our range 3-10) decrease the duration of the window. The level of resistance does not affect the closing time for Scenario A or B but affects the opening times, so higher levels of resistance extend the duration of the window.

Discussion
The emergence and spread of chloroquine resistance, and subsequently sulfadoxine-pyrimethamine resistance has killed millions of people over past 30 years. With the increasing deployment of artemisinin combination treatments containing slowly eliminated partner drugs there is concern that these valuable drugs may also be lost to resistance. The de-novo emergence of resistance to antimalarial drugs resulting from genetic mutations is a rare occurrence. The genetic event is most likely to occur at the peak of infection, when parasite numbers are greatest (23). But this is also when antimalarial drug levels are highest, so only highly resistant parasites will survive correct dosing. Single point mutations in the gene encoding cytochrome B conferring atovaquone resistance are an example of a single step conferring high level resistance, but for the other drugs’ resistance mechanisms the genetic event usually confers low level resistance (p≤10) (24). But if the patient receives inadequate or substandard treatment, malabsorbs or vomits the medication (all of which are relatively common in practice), or has an unusually large volume of distribution for that particular compound, then low blood concentrations may result. These concentrations may be below the MIC for the resistant mutant, and therefore allow its growth. In order for resistant parasites then to spread the de-novo resistant parasite or parasites must multiply sufficiently to generate enough parasites to produce transmissible gametocyte densities (>5/µL of blood). The resistant mutants will also have to contend with immune responses, directed mainly against the variant surface antigens (mainly PfEMP1) expressed on the exterior of the infected erythrocytes. This reduces the selection probability. Stable resistance selection in a single
passage has been demonstrated conclusively for pyrimethamine and atovaquone resistance in *Plasmodium falciparum* infections in humans. So clearly this specific immune response is not very efficient in reducing the selection of resistance. The relationship between parasitaemia, dose, and selection probability was elegantly established for pyrimethamine resistance in experimental infections with the Kampala strain of *Plasmodium falciparum* by Martin and Arnold (12). Combinations reduce the probability of de-novo selection because if a parasite occurs which is resistant to one component, it should be killed by the other. Combinations therefore protect each of the partner drugs (13, 24).

Rapidly eliminated drugs such as the artemisinin derivatives cannot protect their partner drugs once blood concentrations have fallen below their MIC. The artemisinin derivatives are eliminated in hours. Thus a newly acquired infection may encounter subtherapeutic concentrations during the elimination phase of a partner drug from a previously administered treatment which is unprotected by the artemisinin derivative. In order for these parasites to survive they must encounter concentrations of drug which fall below the MIC (23) before these parasites are all killed. If the drug has liver stage activity then in order for them to survive these stages must encounter sub-MIC concentrations for liver stage activity as well. If resistant parasites are acquired from elsewhere (i.e. a large proportion or all the merozoites produced at hepatic schizogony carry resistance genes) then there is a period, determined by the elimination kinetics of the antimalarial and the relative susceptibilities of the resistant and sensitive parasites (i.e. the magnitude of right shift in the respective concentration-effect relationships), during which resistant but not susceptible parasites may establish a transmissible infection. This “window of selection” has been extensively used to model the emergence and spread of resistance, particularly to sulfadoxine-pyrimethamine (7, 8, 9, 20).

In this study we examined the relationship between the pharmacokinetic and pharmacodynamic properties of the antimalarial drug and the window of selection. It is clear that the window of selection is determined mainly by the terminal elimination half-life of the drug, but it is also affected by the degree of resistance, the blood concentrations of drug achieved, and to a lesser extent by the slope of the concentration-
effect relationship. Resistance often confers a fitness disadvantage, which may be reflected in reduced growth rates. This would have the effect of narrowing the window of selection.

The longer the terminal elimination half-life and the flatter the concentration-effect relationship, the wider the window of selection becomes. In most cases where blood concentrations initially are high relative to the minimum parasiticidal concentration (atovaquone resistance is the notable exception) the window of selection opens one or more 48 hr cycles after stopping antimalarial drug administration. Rapidly eliminated drugs (t_{1/2} less than one day) usually provide no window of selection at all. Artemisinin derivatives are eliminated so rapidly that concentrations decline by more than two thousand fold in 12 hours, so they provide only a few hours of potentially selective concentrations, and thus no window. Quinine can select in at most three post-treatment cycles (if a very high level of resistance were to arise). Drugs which are very slowly eliminated provide a wide window provided that the concentrations in the terminal elimination phase are suppressive. But as resistance increases drugs such as chloroquine and piperaquine with prominent distribution phases and very long terminal elimination phases effectively become shorter half-life drugs with correspondingly shorter windows of selection for progressively higher levels of resistance (24).

If a genetic event conferring resistance occurs in a newly acquired infection (de-novo resistance) then it is most likely that only a single (or very few parasites) are resistant initially. This single parasite must survive, multiply, and generate gametocytes in sufficient numbers to transmit for resistance to spread. But it has up to 100,000 drug-sensitive siblings. It is therefore far behind in the “race” to attain densities sufficient for transmission in competition with its siblings. Furthermore whereas resistant parasites acquired from elsewhere can be selected by blood concentrations exceeding the MIC, de-novo resistant parasites cannot (Figure 1). Together these factors provide a much narrower window of selection opportunity for de-novo resistance (Figure 4) compared with acquisition of resistant parasites from elsewhere. Most importantly for short half-life drugs the window is very brief, or in the case of artemisinin derivatives, non-existent. This has very important implications; resistance to artemisinin can occur only by inadequate treatment.
Acknowledgements

We thank Professor NPJ Day and Professor P Olliaro for advice. This study was a part of the Mahidol Oxford Tropical Medicine Research Unit research programme, funded by the Wellcome Trust of Great Britain.
References


Table 1. Parameters used in the simulation study.

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<th>Pharmacokinetic parameter</th>
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<th>Scenario B</th>
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<td>3, 5, 100,</td>
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<tr>
<td>n</td>
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<td>2, 5, 10</td>
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<td>$t_{1/2}$ (days)</td>
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<td>1 – 20</td>
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<tr>
<td>p</td>
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$a =$ ratio of the maximum drug concentration at time 0, $C(0)$, to the $EC_{50}$ of the drug sensitive parasites; $C(0)/EC_{50}$. The $EC_{50}$ is the concentration in-vivo of antimalarial drug giving 50% of maximum inhibition in vitro.

$n =$ slope of the concentration-effect relationship derived from in-vitro studies

$t_{1/2} =$ antimalarial drug elimination half-life

$p =$ ratio of $EC_{50}$ values for resistant and sensitive parasites

Table 2. The minimum antimalarial half-life (days) needed for a selection window to exist. Scenario A.

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$a =$ ratio of the maximum drug concentration at time 0, $C(0)$, to the $EC_{50}$ of the drug sensitive parasites; $C(0)/EC_{50}$

$n =$ slope of the concentration-effect relationship derived from in-vitro studies

$p =$ ratio of $EC_{50}$ s for resistant and sensitive parasites

$a$ (taken as the positive half-life for which the window of selection opening time is the same as the closing time or as the half-life for which the closing point is positive).
Table 3. The lower boundary for the antimalarial elimination half-life (days) for which the window of selection is at least 12h long (upper number) or 24 h long (lower number). Scenario A.

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a = ratio of the maximum drug concentration at time 0, C(0), to the EC_{50} of the drug sensitive parasites; C(0)/ EC_{50}

n = slope of the concentration-effect relationship derived from in-vitro studies

p = ratio of EC_{50}s for resistant and sensitive parasites.
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<th>Cmax (ng/mL)</th>
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<th>Cmax/IC50</th>
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<th>$p_{IC50}^S$</th>
<th>De-novo resistance: Window A opening</th>
<th>closing</th>
<th>duration (days)</th>
<th>Acquired resistance: Window B opening</th>
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1 – Brockman et al (2) unless indicated otherwise
2 – Hombhanje et al (10)
3 - not modeled as it is largely the common metabolite DHA that is responsible for the antimalarial activity
4+ window durations of less than two days are unlikely to provide any selection.
FIGURE LEGENDS

**Figure 1.** The window of selection for a) resistant parasites acquired from elsewhere. b) de-novo resistance in newly acquired infections exposed to residual antimalarial drug concentrations. Blood concentrations of an antimalarial drug ($t_{1/2} = 1$ week) are shown by the brown line. In 1b the window opens when the usually single de-novo resistant parasite, which emerges from the liver, and its progeny $R^1$ can survive and grow i.e. the blood concentrations have fallen to or below the MIC for this level of resistance. The majority population of the sensitive sister parasites ($S^1$) are eliminated. The window closes at a time when the growth of sensitive parasites ($S^2$) emerging from the liver outstrips growth of the resistant parasites ($R^2$) such that by the time $S^2$ reach $10^8$ there are $<10^7 R^2$ parasites.

**Figure 2.** Relationship between the selection window (vertical line) for scenario A and the drug half-life in case of (a) resistance intensity; $p = 2$; (b) resistance intensity; $p = 4$; (c) resistance intensity; $p = 6$. $n$ denotes slope of the concentration/effect curve; “a” denotes the ratio $C_0/EC_{50}$ and “p” = $IC_{50}^R / IC_{50}^S$. The red arrow points to the half-life value below which no window of selection occurs.

**Figure 3.** Window opening times for scenario B, for (a) resistance intensity =1.5 (full circle); (b) resistance intensity =2 (empty circle); (c) resistance intensity =2.5 (empty triangle). “n” denotes slope of the concentration/effect curve; “a” denotes the ratio $C_0/EC_{50}$. Note that the scale is different in each graph.

**Figure 4.** Windows of selection for scenario A (vertical lines) and scenario B (solid lines) for different half-lives in case of: intensity of resistance $p = 2$, slope $n = 5$, ratio $a = 5$.

**Figure 5.** Relationship between the duration of windows of selection for scenario A and scenario B for intensity of resistance = 2. Each line is for a given value of slope $n$, for all levels of ratio $a$. 
Figure 2 A

Time after drug given (days)

- n=3 a=3
- n=3 a=5
- n=3 a=100

- n=5 a=3
- n=5 a=5
- n=5 a=100

- n=10 a=3
- n=10 a=5
- n=10 a=100

Drug half-life (days)
Figure 2 C

Time after drug given (days)

- n=3 a=3
- n=5 a=5
- n=10 a=100

Drug half-life (days)
Figure 3

Time after drug given (days)

n=3 a=3

n=3 a=5

n=3 a=100

n=5 a=3

n=5 a=5

n=5 a=100

n=10 a=3

n=10 a=5

n=10 a=100

Drug half-life (days)
Figure 5

Duration of the selection window (days) – scenario B

Duration of the selection window (days) – scenario A